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ADAPTATIONS OF MUSCLE GLYCOGEN PHOSPHORYLASE TO EXERCISE AND TRAINING

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "ADAPTATIONS OF MUSCLE GLYCOGEN PHOSPHORYLASE TO EXERCISE AND TRAINING" submitted by Michael Anthony Booth in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Human subjects were exercised on a bicycle engometer, both pre- and post-training, at work loads designed to induce 70% and 100% MVO_2. A control group was used for comparison with the pre-training subjects. A biopsy needle was used to take initial, fatigue and recovery samples from the vastus lateralis muscle of the thigh and these samples were assayed for changes in glycogen content and phosphorylase enzyme activity.

Training produced an adaptive response in muscle, characterized by biosynthesis of the specific phosphorylase protein, but a similar change in total phosphorylase was not observed during single bouts of either short maximal or prolonged submaximal exercise. The trained state was always associated with higher concentrations of active enzyme, phosphorylase *a*, but the percentage of the enzyme in the *a* form was unchanged due to the proportional increase in the levels of total phosphorylase.

Within any one group, no significant change in phosphorylase *a* was observed in response to single exercise bouts, but the sensitivity of the phosphorylase interconversion mechanism to the experimental techniques and procedures did not permit valid conclusions to be drawn with respect to the relative importance of the *a* and *b* forms in glycogenolysis or the onset of fatigue.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

STATEMENT OF THE PROBLEM

Introduction

Glycogen phosphorylase* is the principal enzyme involved in the breakdown of glycogen in both muscle and liver (16), catalyzing the initial conversion of glycogen to G-1-P. The pathway from glycogen to lactic acid is a multi-enzyme system and the rate at which energy can be produced from glycogen is determined by the activities of certain enzymes which act as "directional flow values" (26:227) within the system. The phosphorylase enzyme limits the maximal rate at which energy can be produced from glycogen because it catalyzes the first reaction in the sequence (42, 80). The significance of the phosphorylase enzyme in exercise is clearly illustrated by McArdle's disease, in which muscle phosphorylase is almost completely lacking (59, 71). Patients suffering from this rare disease cannot make use of their muscle

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* Abbreviations used in this thesis are: glycogen phosphorylase = α-1, 4-glucan: orthophosphate glucosyltransferase, EC 2.4.1.1; glycogen synthetase = uridine diphosphate glucose: α-1, 4-glucan-, 4-glucosyltransferase, EC 2.4.1.11; debranching enzyme = α-1, 6-glucosidase; branching enzyme = α-1, 4→1, 6-transglucosidase; UDPG = uridine diphosphate glucose; AMP = adenosine monophosphate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; cyclic AMP or 3,5'-AMP = 3,5'-adenosine phosphate; PCr = phosphocreatine; G-1-P = glucose-1-phosphate; G-6-P = glucose-6-phosphate; G-6-Pase = glucose-6-phosphatase; PFKase = phosphofructokinase; P-b-Kase = phosphorylase-b-kinase; P_i = unorganic phosphate; DPN = NAD = diphosphopyridine nucleotide.

glycogen stores, and consequently, have a considerably reduced physical working capacity.

Phosphorylase itself has been shown to exist in interconvertible, a and b forms (46, 17), which differ in molecular weight and activity (45, 76). The breakdown of ATP to AMP was found (55, 60, 61) to be the stimulus for phosphorylase b activity, whereas human phophorylase a was 85 percent active independently of AMP levels (85). Consequently, only the a form of the enzyme has the potential to degrade glycogen in resting or aerobic skeletal muscle, in which the ATP concentration is high and AMP levels are low (60, 61). However, studies with animals have revealed that phosphorylase is almost entirely in the b form in resting muscle and that tetanic stimulation (18, 19, 74) or epinephrine administration (18, 19, 35, 45) causes a rapid conversion to the a form.

Knox, Auerbach and Lin (43) reviewed the effects of a wide variety of physiological stimuli on enzyme activity and described two kinds of enzymic adaptation. The first kind of adaptation is a change in the concentration of the enzyme in the cell, and implies either synthesis or degradation of the specific enzyme protein (29, 36, 43). Adaptive changes in concentration can be inferred from activity measurements, provided that assay conditions are suitable (43:168). If, however, no change in activity is observed under assay conditions, the possibility still exists that a second kind of adaptation has taken place *in vivo*. This

second type of enzymic adaptation has been termed the kinetic response (43) or the overall metabolic potential of the environment in the cell (29). Kinetic factors probably play some role in momentary adjustments of metabolic rate before enzyme biosynthesis has time to occur (43:176).

From the foregoing discussion, it might be expected that physical training or conditioning would induce adaptation in terms of concentration changes, whereas unique exercise bouts, especially of short duration, would produce adaptations of a kinetic nature.

If phosphorylase a could be assumed to be the only active form of the enzyme at any stage during exercise, then the assayed activity of phosphorylase a should reflect the phosphorylase influence upon the rate of glycogenolysis. If the phosphorylase a concentration and the rate of glycogenolysis do not change proportionally, then evidence exists for consideration of kinetic concepts such as the following, in order to explain the reaction rates observed in vivo:

- the activation or inactivation of phosphorylase b by AMP and ATP levels (60, 61)
- compartmentation within the cell and the availability of the substrate to the enzyme (34, 40)
- the concentration of the debranching enzyme (1, 16)
- the degree of cooperation of other enzymes in the glycolytic chain in the removal of end-products (42)
- the local pH and temperature (46, 56).

Statement of the Problem

The purpose of this study was to obtain muscle biopsy samples from human subjects and to analyze these samples for possible adaptations of the phosphorylase enzyme. The samples were to be obtained during exhaustive exercise of short and long duration in trained and untrained subjects.

Consideration was to be given to the following adaptations:

(1) Changes in the total concentration of phosphorylase present per unit weight of muscle, measured as the summed activities of the *a* and *b* forms in an optimum-condition assay situation.

(2) Changes in the amount of the active form of the enzyme, phosphorylase *a*, expressed both in absolute terms and as a percentage of the total enzyme concentration.

Rationale Behind the Study

The well-established fact that skeletal muscle tissue can undergo extreme changes in metabolic rate during exercise, is sufficient indication in itself, that kinetic responses of enzyme systems do occur. However, the postulate of an increase in kinetic efficiency with training is still debatable (27, 29, 31, 32, 36, 65, 82, 83).

Considerable disagreement exists also with respect to the adaptive biosynthesis of enzyme protein in response to exercise (13, 41) and training (27, 29, 31, 32, 36, 82,

83). However, the abundance of positive responses obtained, to other physiological stimuli (43), suggests that such an adaptation is likely to occur if the exercise is sufficiently severe and prolonged.

Most research into the phosphorylase enzyme system has been directed at an understanding of the nature and importance of the complex enzyme inter-conversion system. Consequently, a wealth of information is available on epinephrine administration (18, 19, 35, 45) and tetanic electrical stimulation (18, 19, 74) of muscle *in situ*, but only one study (13) was found which examined the nature of the adaptive responses to maximal or submaximal exercise. In addition, very few studies (29, 82, 83) have considered the phosphorylase adaptation to training schedules.

The use of human subjects in enzyme studies has not been practically feasible until the recent development of the needle biopsy technique (11). This procedure has been used successfully to determine human muscle glycogen content (23, 38, 49, 70) and one report has appeared in which the technique has been adapted to enzyme studies in exercise (41).

In view of recent studies which have implicated muscle glycogen as an important energy source in maximal (49) and submaximal (23, 38, 49, 70) exercise, very little consideration has been given to the factors which may be limiting the production of energy from the substrate. No previous investigations have been conducted on the phosphorylase enzyme responses to similar exercises or to training in human subjects.

CHAPTER II

REVIEW OF THE LITERATURE

Pathways of Glycogen Metabolism

ATP is the immediate source of all energy for muscular contraction (39). When one, or both, of the high-energy phosphate bonds of the ATP molecule are split, ADP and AMP, respectively, are formed. The total ATP stores of muscle, however, are sufficient for only about 0.5 seconds of work at a maximal rate (79:749). The PCr stores can be used to resynthesize ATP according to the equation, PCr + ADP $\leftrightarrows$ Cr + ATP, but the total utilization of these resources could only extend work time by a few seconds (79:750). Anaerobically, the principal method for resynthesis of ATP, from AMP and ADP in muscle, is the conversion of glycogen or glucose through pyruvic acid to lactate. If oxygen is available, a great deal of ATP can be regenerated by oxidation of pyruvic acid and fat derivatives.

G-6-P and pyruvic acid are important components in the glycolytic sequence (Figure 1) because they occupy primary junction sites. At the G-6-P junction (79:367), pathways enter from glycogen via the phosphorylase enzyme system, and from blood glucose catalyzed by hexokinase. The major output channel from this point is the Embden-Meyerhof pathway through to pyruvic acid. This pathway yields only a small amount of energy per glucose molecule,

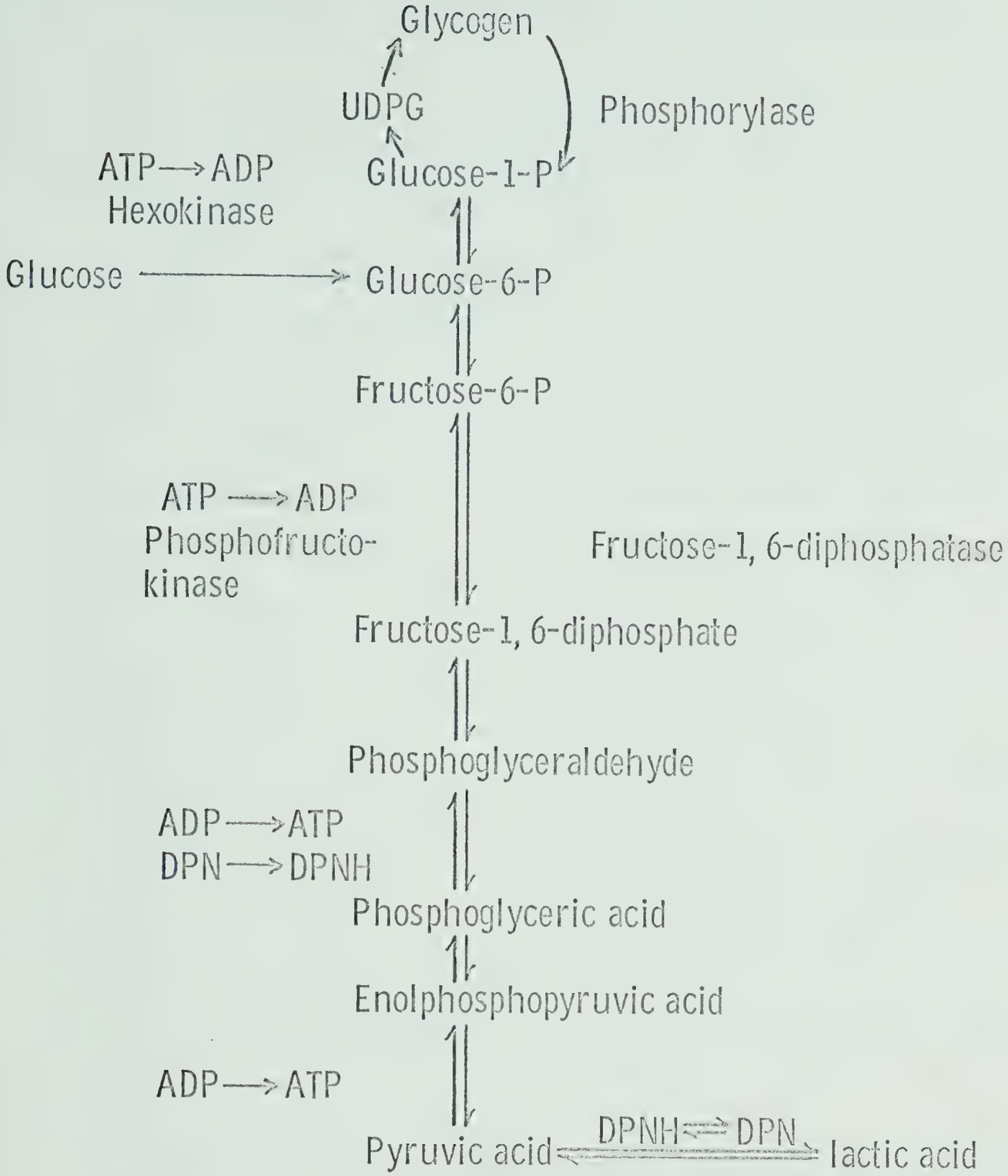


FIGURE 1

PATHWAY FOR THE ANAEROBIC RELEASE OF ENERGY

FROM GLYCOGEN IN SKELETAL MUSCLE

From Ganong (25: 223)

$$\text{glucose} + 2\ \text{ADP} + 2\ P_i \rightleftarrows 2\ \text{lactic acid} + 2\ \text{ATP},$$

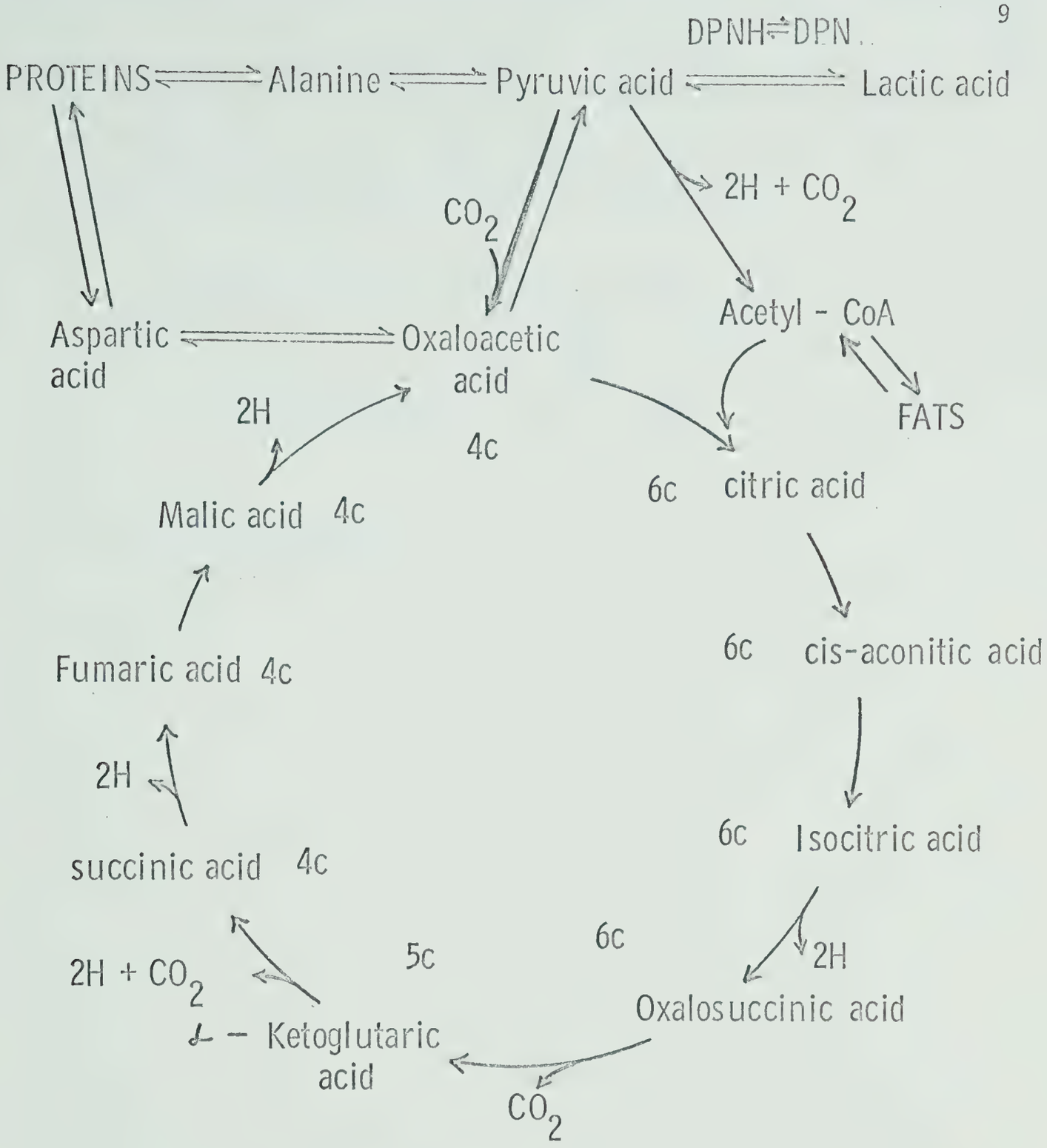
but this process is entirely anerobic (26:226).

An alternative degradative pathway, the hexosemonophosphate shunt, leaves from the G-6-P junction, but this pathway requires oxygen. In the liver, the shunt pathway provides about 30 percent of the total energy but no evidence exists for its function in skeletal muscle (79:390).

Pyruvic acid (79:378) occupies the second major junction site. Pyruvic acid molecules can enter the mitrochondria and, if oxygen is available, can be oxidized together with the pool of short-chain intermediates from fat and protein catabolism. The enzymes of the citric acid cycle (Figure 2) and the flavoprotein-cytochrome system are responsible for these oxidative reactions (26:219). The end-products are carbon dioxide, water, and a large amount of energy in the form of ATP for muscle contraction. This chain of reactions through pyruvate and the citric acid cycle produces the maximum energy yield from a unit of glucose (79):

$$\text{glucose} + 6\ O_2 + 38\ \text{ADP} + 38\ P_i \rightarrow 6\ CO_2 + 6\ H_2O + 38\ \text{ATP}$$

If oxygen is not available, the pyruvic acid must be converted to lactic acid, or anaerobic energy production will quickly cease. This conversion yields no additional energy, but the reaction is accompanied by the reformation of DPN (26:226). DPN is in short supply in the muscles and



Overall reaction:

Pyruvic acid + oxygen ⟶ carbon dioxide + water

$C_3H_4O_3 + 2\ 1/2\ O_2 \longrightarrow 3\ CO_2 + 2\ H_2O$

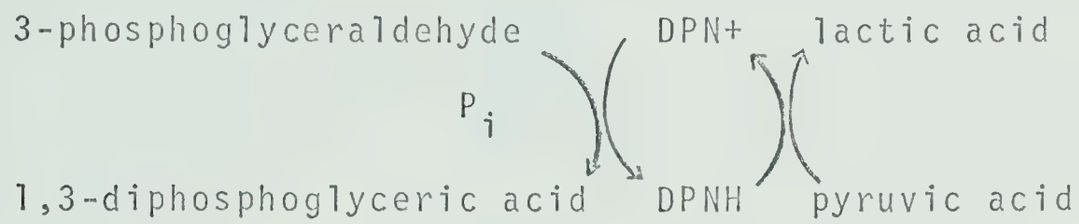
15 ADP ⟶ 15 ATP

FIGURE 2

THE CITRIC ACID CYCLE

From Ganong (25: 222-224)

is required for the conversion of phosphoglyceraldehyde to phosphoglyceric acid in the glycolytic sequence. The relationship has been summarized as follows (79):



Rate-Limiting Reactions in Glycogen Metabolism

In a multi-enzyme system, some reactions may be essentially irreversible. These reactions proceed in one direction under the influence of one enzyme, and in the opposite direction under the influence of another enzyme. Factors which regulate metabolism act directly or indirectly at these "directional flow valves" (26:226). In the sequence of reactions from glycogen to pyruvic acid in muscle cells, the reactions (Figure 3) catalyzed by the phosphorylase and PFKase enzyme systems are potentially rate-limiting (19, 26:226, 42, 57, 79:381, 80). Phosphorylase catalyzes the first reaction in glycogenolysis and so should determine the maximal rate of the system (42). PFKase can actually control the rate at which glycolysis proceeds within the limits set by the initial phosphorylase activity (19, 42, 57, 80).

Several authors, however, have reported a high degree of coordination in the action of these two enzymes (42, 57). Karpatkin, Helmreich, and Cori (42) obtained a maximum rate of lactate production of 130 times the resting level when

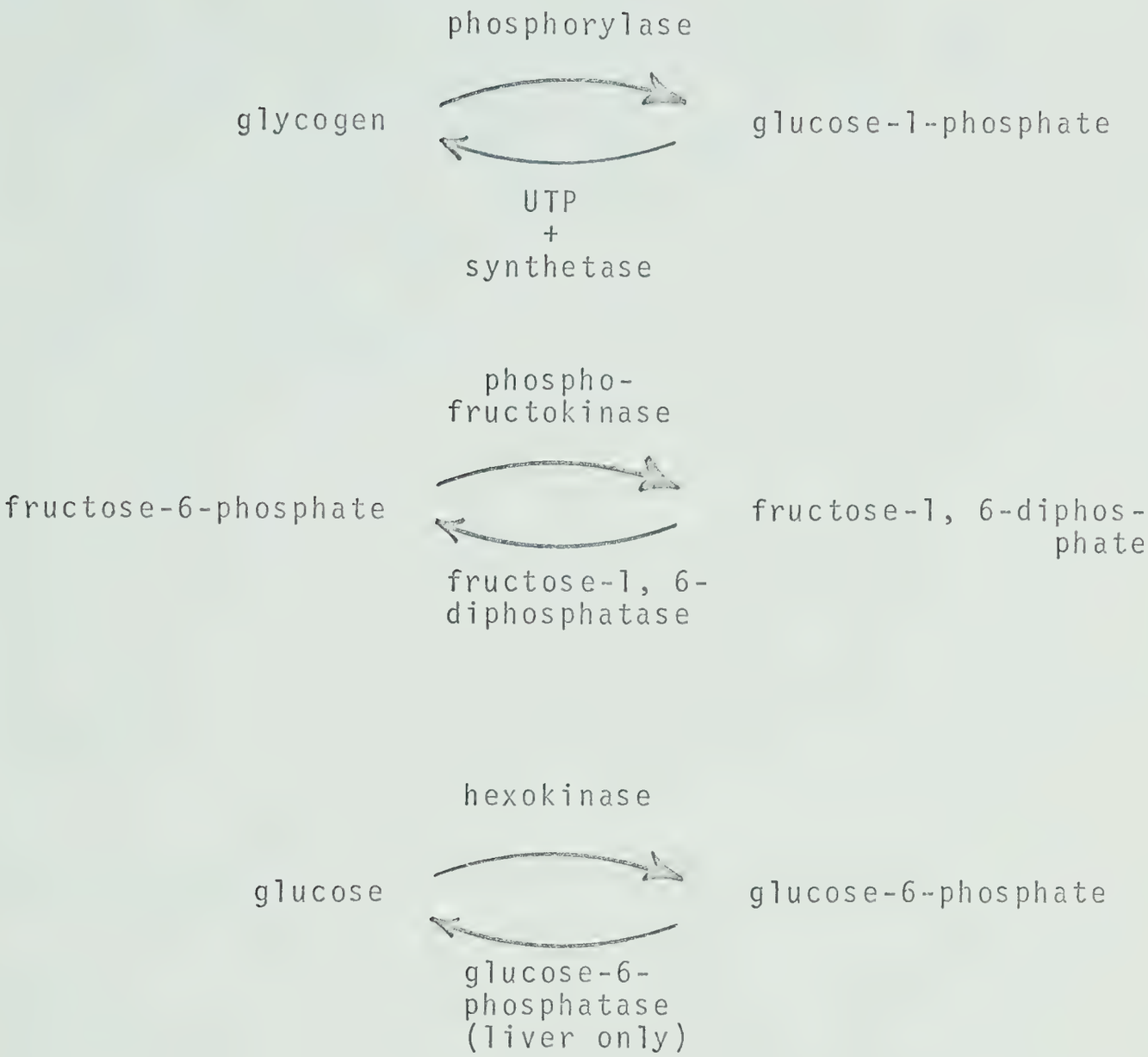


FIGURE 3

DIRECTIONAL FLOW VALVES IN GLYCOLYSIS

From Ganong (25:227)

they electrically stimulated isolated frog sartorius muscle and they remarked that this was not the maximal capacity of the system. Measurements of G-6-P and lactate levels at periodic intervals revealed that the two enzymes remained in step over a wide range of flow rates through the glycolytic system. Mansour (57), in a review of recent research, stated that the phosphorylase and PFKase enzyme systems appeared to be adapted to work in tandem. Both enzyme systems have been activated by cyclic AMP (76) and by physiological conditions such as anoxia and muscular contraction (57). In addition, the activities of both enzyme systems have been shown to be inhibited by the presence of ATP and enhanced by high concentrations of AMP (57).

Functional Relationships of Enzymes in Glycogenolysis

The enzyme, phosphorylase, cannot act by itself to completely degrade glycogen. Nor can the formation of the polysaccharide molecule be accomplished entirely be glycogen synthetase. Both of these enzymes act only at the α-1, 4-glucosidic linkages (56, 76). Complete breakdown of glycogen can be accomplished rapidly by phosphorylase in conjunction with debranching enzyme, amylo-1, 6-glucosidase (56, 76). This debranching enzyme transfers the glycogen side chains from α-1,6 to α-1,4 linkages and so makes more substrate available to phosphorylase (1). Another transferase enzyme, oligo-1, 4→1, 4-glucantransferase, may be capable of substituting for phosphorylase, but

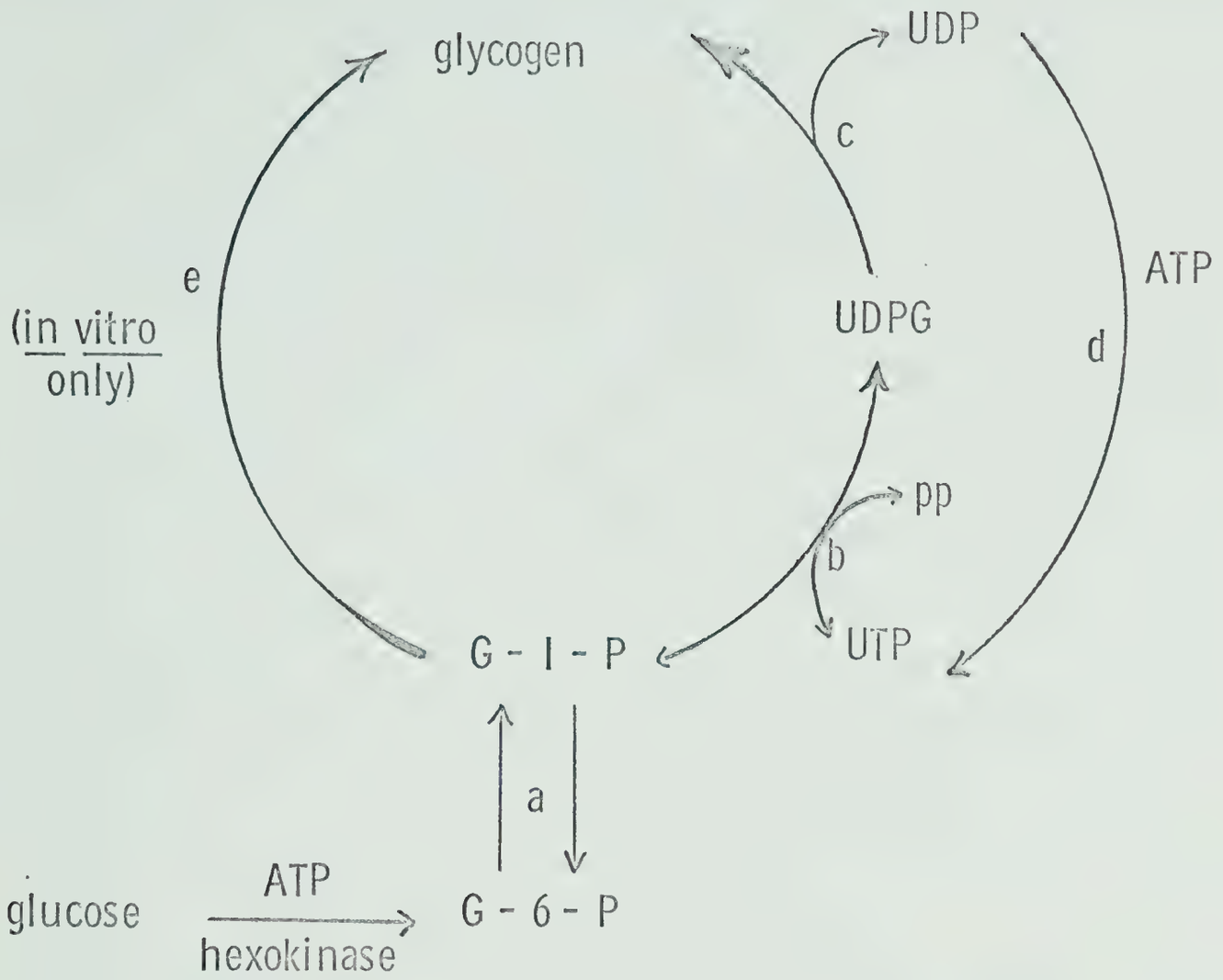
the rate would probably be so slow as to have no significance *in vivo* (1). Several glucosidases which may be associated with glycogen breakdown, are also present in muscle, but their significance is not yet fully understood (68).

Glycogen synthetase acts in conjunction with branching enzyme to synthesize the complex glycogen molecule (16). The pathway is via G-1-P and UDPG (Figure 4) and has been expressed by French (24) as,

$$UDPG + G_n \xrightarrow[\text{synthetase}]{\text{glycogen}} G_{(n+1)} + UDP$$

Normally, in muscle, the synthetase and phosphorylase systems act simultaneously and in balance, such that glycogen is turned over a constant rate, with a half-life of approximately four days (76). However, if rapid breakdown is required, the physiological stimuli to activity must have opposite effects on the two enzyme systems. Such effects have been demonstrated in response to hypoxia (42, 61, 76), cyclic AMP and epinephrine (10, 18), and G-6-P (61). Insulin, which activates synthetase (50) has not been found to influence phosphorylase (50).

Staneloni and Piras (74) applied successive ten-second tetanic stimuli, separated by four minutes of recovery, to rat skeletal muscle *in situ* and measured the changes in phosphorylase and synthetase activity. The results revealed a very definite reciprocal relationship between the activities of the active form of the two enzymes, phosphorylase *a* and



a. Phosphoglucomutase
b. UDPG - pyrophosphorylase
c. UDPG - glycogen transferase (synthetase)
d. UDP - kinase
e. Phosphorylase

FIGURE 4

PATHWAYS OF SYNTHESIS OF α-1, 4' LINKAGES OF GLYCOGEN. From Stetten and Stetten (75:515)

synthetase I, to prevent unnecessary recycling of glucosyl residues. No changes in total enzyme concentrations were detected.

Canal and Frattola (13) also found reciprocal changes in the active forms when rats were made to swim for six hours, but the changes were in the opposite direction to those reported by Staneloni and Piras (74) in response to tetanus. Compared to the control rats, the fatigued animals were lower in phosphorylase a, though not significantly, and higher in synthetase I. The total concentrations of both enzymes were also higher in the group subjected to the prolonged exercise, but again the phosphorylase change was not significant. After six hours of recovery, the positions were reversed.

Distribution of Glycogen and Phosphorylase in Muscle Cells

Glycogen is a complex, branched polymer of glucose and is the principal storage form of carbohydrate in the body, the muscles and liver being the major depots. Phosphorylase (52) and synthetase (58) have been shown to exist in association with glycogen, in the particulate granules of the cytoplasm (79:50), but are partially released from it during stimulation (74). Sigel and Pette (73) used histochemical staining techniques to localize phosphorylase, and other glycolytic enzymes in the rabbit, to the sites of the isotropic bands, which correspond to the cross-striations in muscle. This suggested either a location within the inter-

filamentary sarcoplasm of the actin filaments or an association with the transverse sarcotubular system (73).

Phosphorylase, however, has not been found to be equally distributed in all fibers (22, 44, 47), nor in different muscles (22, 47, 67), nor in different regions of the same muscle (44, 47, 67). Rather, muscle fibers have been recently classified by both histochemical (47, 67) and biochemical (9) techniques, into a spectrum of types in which red and white fibers are the two extremes. Complex muscles like gastrocnemius and quadriceps have revealed a mozaic of enzyme patterns (Figure 5) (44). Gastrocnemius muscle was composed primarily of white fibers, while soleus was predominantly red (67). Red fibers were found to be high in esterase and oxidative enzyme activity, and in mitochondrial and myoglobin contents (22, 67). White fibers had high concentrations of glycogen (48), phosphorylase and glycolytic enzymes (47, 67). Despite this diversity in muscle, several researchers have reported that human muscle glycogen levels, determined from needle biopsy specimens, were consistent in the two halves of the same biopsy specimen (38), in biopsies taken from proximal and distal parts of the same muscle (3) and in biopsies from corresponding muscles on the two sides of the body (3, 12). Muscle glycogen was different, however, in different muscle groups (38) and in red and white fibers (48).

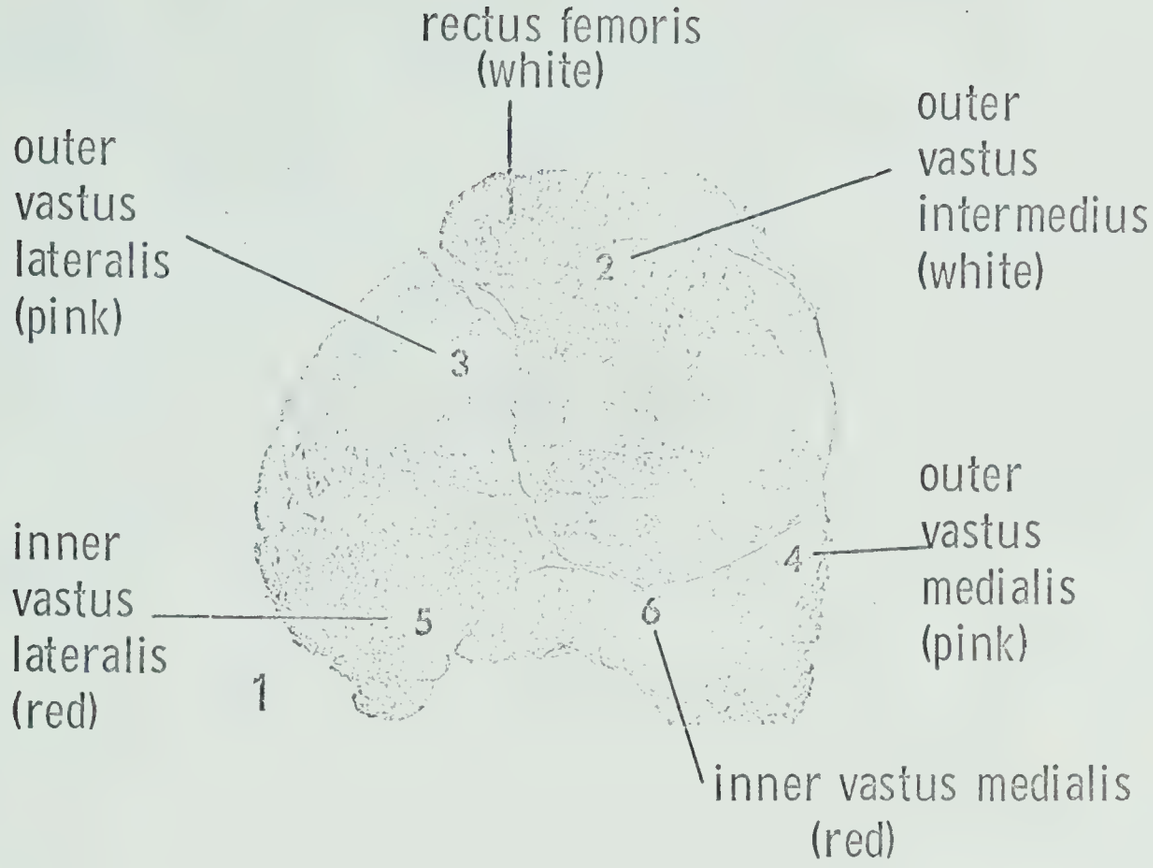


FIGURE 5

REGIONS OF THE QUADRICEPS OF RAT

Based on histochemical detection of enzyme activity patterns. From Kowalski et al. (44)

Theoretical Significance of Glycogen as an Energy Source

Two important factors might be expected to determine the priority usage of fuels by active skeletal muscle, the availability of extramuscular energy supplies and the availability of oxygen for combustion of fuels. If, at any time, these supplies become inadequate, the muscle must become more dependent upon the anaerobic oxidation of intramuscular fuels. Glycogen is the only intramuscular substrate which could pro-

vide significant amounts of energy under these circumstances. Hypothetically, then, the body should place a first priority upon muscle glycogen as a reserve energy supply, to be spared for emergency situations. Glycogen degradation would, then, be most rapid in situations where blood supply to the cell was inadequate, oxidative capacity of the cell was limited or extramuscular energy stores were exhausted.

Some support for this theory is derived from the particularly rapid rates of glycogen breakdown observed in the initial stages of muscular activity (14, 80), when the body is struggling to establish a new steady state. At rest, or in very light work, the stores of glycogen in the muscles are not depleted, fatty acids, and glucose extracted from the blood, are the major sources of energy (8, 79:754). As exercise becomes more intensive, glycogenolysis becomes increasingly more rapid (17, 49).

Fitness level (29) and diet (2, 12, 70) are two other factors which have been found particularly influential in determining the rate of breakdown.

The Phosphorylase Enzyme System

Skeletal muscle glycogen phosphorylase has been the subject of extensive investigation over the past half century. These investigations have revealed a very complex enzyme system (Figure 6) involving one or more enzymes, as well as coenzymes, activators, and inhibitors.

The original *in vitro* studies suggested that phos-

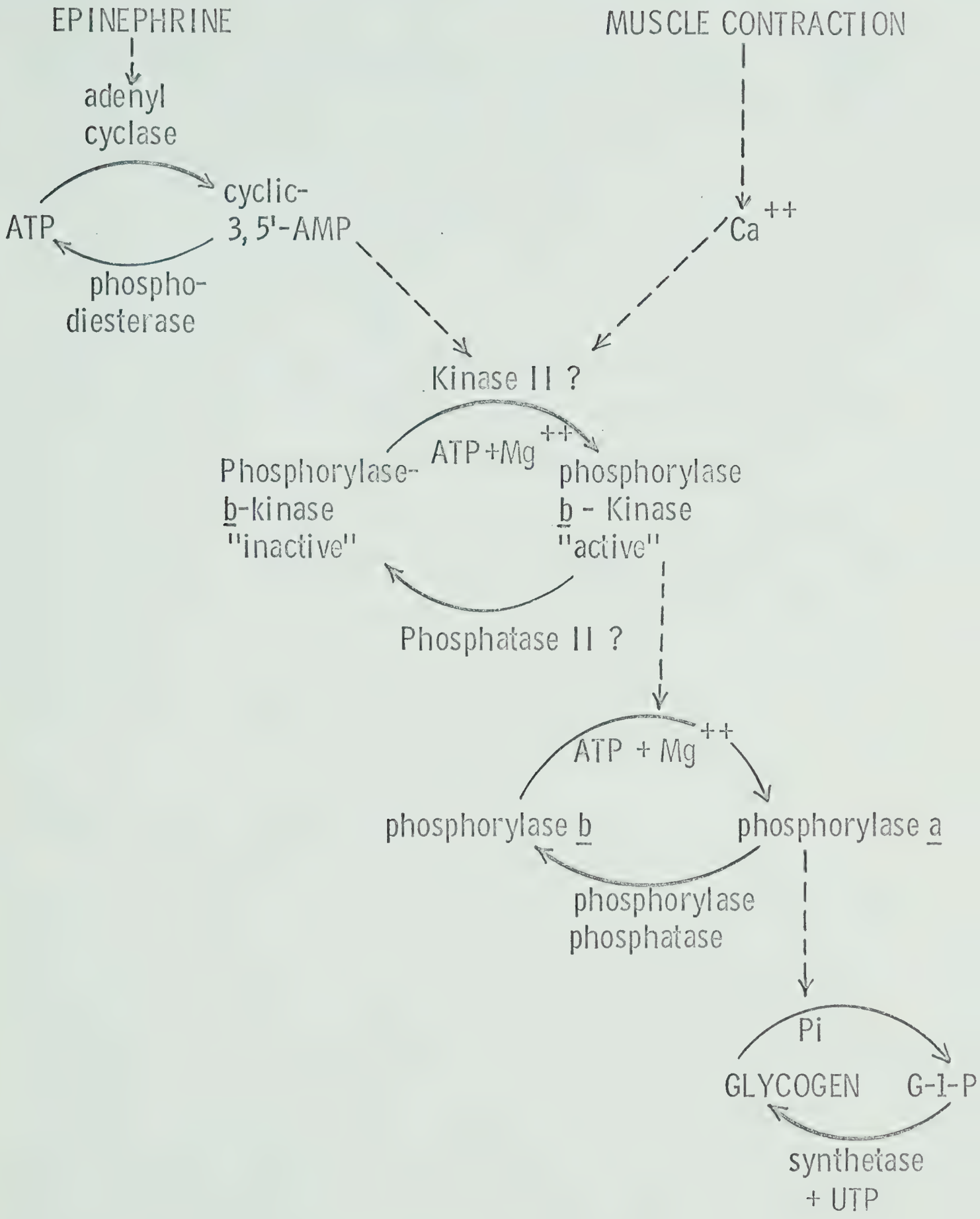


FIGURE 6

THE INNER PHOSPHORYLASE ENZYME SYSTEM
AND ITS ACTIVATION BY EPINEPHRINE
OR MUSCULAR CONTRACTION

phorylase was involved primarily in the synthesis of glycogen (16). However, in the late 1950's the glycogen synthetase enzyme was detected and a pathway for synthesis of glycogen through UDPG was hypothesized (52, 78). Evidence then began to accumulate for a solely degradative function for phosphorylase *in vivo*. At the 1964 CIBA Foundation Symposium on the control of glycogen metabolism, it was agreed that under the physiological conditions which exist in the muscle, phosphorylase would have a predominantly, if not entirely, degradative function, while the synthetase system would be the principal pathway for glycogen formation. *In vitro*, however, the phosphorylase reaction is readily reversible and, in fact, the reverse reaction is used as a basis for most activity measurements (17, 69).

Phosphorylase Activation

Phosphorylase has been found to exist in *a* and *b* forms (17, 46), with phosphorylation, and a doubling of the molecular weight, being involved in the conversion from *b* to *a* (45, 76). The *a* form was found capable of degrading glycogen even in resting muscle, but was present in only very small concentrations (18, 20). The *b* form was dependent upon high AMP levels for activity and was therefore inactive in resting or aerobic muscle (19, 60). The conversion from the inactive to the active form of the enzyme has been termed activation, and was shown to be under enzymatic control (45, 46). The enzyme catalyzing the *b* to *a* reaction was referred

to as phosphorylase-b-kinase (46) while the deactivating enzyme was phosphorylase phosphatase, previously called PR-enzyme (15, 17).

Activity of Phosphorylase b

Early researchers reported that the concentrations of AMP required for phosphorylase b activity, were far in excess of those known to occur in muscle (18, 79) so phosphorylase a was postulated as the only active form of the enzyme. Evidence has been accumulating, however, that phosphorylase b under physiological conditions, may be capable of catalyzing the breakdown of glycogen. Lyon and Porter (55) exercised a strain of mice which lacked P-b-Kase in muscle and so could not convert phosphorylase b to phosphorylase a. They found that these I-strain mice could perform endurance swimming exercise as well as could the normal mice, and that glycogen supplies were being utilized for the exercise. This suggested that phosphorylase b itself could catalyze glycogen breakdown in muscle, and that conversion to phosphorylase a was not necessary for this type of exercise.

Recent work by Morgan and Parmeggiani (60, 61) has provided an explanation for these contradictory findings. In vitro experiments with perfused rat hearts (60) and skeletal muscle of rabbits (61) led to the conclusion that phosphorylase b could exert activity. High AMP concentrations were found to increase the activity of phosphorylase b, while ATP and G-6-P acted as inhibitors. In resting or aero-

bic muscle, where phosphorylase was almost entirely in the b form, ATP and G-6-P concentrations were sufficiently high to inhibit the activating effect of the low AMP levels that were present. Consequently, glycogen levels were stable. In anaerobic muscle, however, the reverse situation prevailed. ATP and G-6-P levels were lowered while AMP levels were increased sufficiently to overcome the inhibitory effects. There was also an increase in phosphorylase a, and the results suggested that phosphorylase a and phosphorylase b were almost equally responsible for the glycogen breakdown that occurred in the anaerobic muscle.

Activation of Phosphorylase-b-kinase

P-b-Kase, like phosphorylase, has been found to exist in two interconvertible forms which differ in activity (46). These two forms have been termed, simply, P-b-Kase active and inactive (45). The active form is required for phosphorylase b to a conversion but the inactive form predominates in the resting muscle. Kinetic analysis (22, 46) has indicated that kinase activation, rather than phosphatase inhibition is responsible for observed increases in phosphorylase a levels during muscle stimulation. Activation of P-b-Kase, like the activation of phosphorylase, is a phosphorylation process (46), but the mechanism has proved extremely complex (Figure 6). Both epinephrine administration and muscle contraction have been found to activate P-b-Kase (45), but the mechanisms were not the same.

Effects of Epiniphrine on Phosphorylase Activity

Epinephrine has long been known to stimulate glycogenolysis in liver and muscle (17), and several researchers (19, 33, 63) have detected sharp increases in the percentage of phosphorylase *a* in the tissues after epinephrine administration. Epinephrine appears to activate adenyl cyclase, in the cell membrane (66, 76), to convert ATP to cyclic 3,5'-AMP. The cyclic nucleotide, in turn, appears to activate an enzyme, Kinase II (45), which has so far defied identification. This kinase then activates the P-*b*-Kase (45) which, in turn, activates phosphorylase. Cyclic AMP is not essential for the reaction to proceed, but it does act as an accelerator (46). Evidence from drug blockade studies (35, 54, 72) suggests that the action of epinephrine is via β-adrenergic receptor sites and adenyl cyclase, possibly in the cell membrane (66).

When compared to the effects of direct electrical stimulation upon the phosphorylase *a* levels of skeletal muscle, epinephrine was found to act much more slowly and less potently (18, 19, 33, 42, 72). Whether epinephrine activation plays any significant role in exercise is thus doubtful. Also, epinephrine was not found effective in activating G-6-Pase in liver (72) or PFKase (42, 57), the other major rate-limiting step in glycolysis. The collated evidence suggests that epinephrine, as an activator of phosphorylase, is secondary in significance to glucagon, in the liver, and to mechanical contraction, in the muscle.

An interesting observation, however, was made by Lyon and Porter (55) in their I-strain mice. Epinephrine did initiate glycogenolysis despite the genetic absence of P-*b*-Kase. Lundholm *et al*. (54) have suggested, as a possible explanation, that epinephrine can increase phosphorylase *b* activity by lowering the levels of ATP in the tissue.

Effects of Muscular Contraction Upon Phosphorylase Activity

Wilson *et al*. (80) found that, in a 30 second tetanus of cat gastrocnemius muscle, 90 percent of the hexose entering the glycolytic chain of reactions stemmed from glycogen rather than from blood glucose. Moreover, the concentrations of nucleotides AMP, ADP, and ATP were such that phosphorylase *b* could not have been active. These findings, together with the extremely rapid glycolytic rates observed *in situ* (17, 20, 42) have led to the conclusion that the phosphorylase *b* to *a* conversion assumes particular importance when skeletal muscle is subjected to high-frequency stimulation.

Danforth, Helmreich and Cori (19) applied electrical stimuli to isolated frog sartorius muscle and, with improved techniques, were able to trace rapid changes in phosphorylase activity. The proportion of the total activity attributable to phosphorylase *a* rose more quickly and settled at a higher steady state level, as the frequency of stimulation was increased. At 35 shocks per second, the percentage of phosphorylase *a* rose from 2.8 to 90 percent after only three seconds. Complete recovery from the tetanus was

obtained in about 60 seconds. Other researchers (17, 18) have found a similar relationship between stimulation frequency and rate of conversion to phosphorylase *a*. In none of these studies, however, did total phosphorylase (*a* + *b*) change significantly.

Further evidence for the importance of phosphorylase *a* in high frequency stimulation was provided by Danforth and Lyon (20), who found a rate of glycogenolysis in normal mice which was twice that in the phosphorylase-*a*-deficient I-strain mice. Phosphorylase *a* was concluded to be the most efficient form of the enzyme during tetanus.

The findings (18, 19, 72) that electrical stimulation produced a much faster activation than did epinephrine suggested some alternative method of activation other than that mediated by cyclic AMP. The correlation between activation and stimulation frequency led researchers to suggest that activation was possibly geared to the contraction process itself (42, 57). The finding that Ca^{++} ions can activate P-*b*-Kase *in vitro* (46), has provided a strong link with the contraction process but the majority of researchers have failed in attempts to reverse the reaction (21, 46). A recent study, however, has reported reversal *in vivo* (62). If a true reaction reversal can be demonstrated *in vivo*, the link with the contraction process will be substantiated.

Effects of Submaximal Exercise Upon Glycogen and Phosphorylase

Ahlborg (2, 3) Hultman (38), and Saltin and Hermansen (70) used the muscle biopsy technique to determine the glycogen content of human quadriceps during prolonged exercise on a bicycle ergometer. Work-loads were set to induce oxygen uptakes between 60 percent and 80 percent of maximal. The glycogen levels were reduced progressively throughout the exercise, but breakdown rate appeared to decrease slightly as exercise continued. Severe, but not absolute, depletion was always evident at the time of fatigue in these studies.

No comparable studies have been conducted to determine associated enzyme changes, but Canal and Frattola (13), have investigated the phosphorylase changes induced in muscles of rats by six hours of swimming. Phosphorylase levels in rats killed at rest, after exercise, and after six hours of recovery were compared. No measurements were made at intermediate times. Phosphorylase *a* levels were lower in the fatigued rats than in either resting or recovery groups, but the differences were not significant.

When low frequency electrical shocks were applied to muscles of various animals *in situ*, phosphorylase *a* activity was found to rise to low levels initially, then to gradually decline as stimulation continued. At the point of fatigue, levels of the *a* enzyme were very low, a phenomenon observed also by Kugelberg and Edstrom (47) using histochemical techniques. Glycogen depletion rates, and

possibly even fatigue, could be explained by these phosphorylase a changes.

Lyon and Porter (55), however, have produced evidence for activity of phosphorylase b in submaximal work. I-strain mice, which could not produce phosphorylase a, were capable of swimming for more than one hour, whereas normal mice became exhausted within 30 minutes. Moreover, the rate of glycogenolysis was higher in the I-mice. The longer work-times observed could be merely a reflection (38) of the extremely high initial glycogen levels in the I-strain animals (55), but the fact remains that phosphorylase b was capable of activity sufficient to produce the required amount of energy from glycogen. Total phosphorylase concentration was the same in both groups.

None of the studies reviewed (13, 17, 19, 74) reported any significant change in the total phosphorylase concentration of muscle during prolonged exercise. In the six hour swim study by Canal and Frattola (13), however, the total phosphorylase activity in the fatigued rats was high, suggesting that adaptive enzyme biosynthesis may have proved significant if a more powerful experimental design had been employed.

Effects of Maximal Exercise Upon Phosphorylase Activity

Glycogen breakdown, in man, has been found to occur more rapidly during brief maximal exercise than during prolonged submaximal work (49, 70), but glycogen stores

were still high at the point of exhaustion (49, 70) because of the short exercise duration. Cori (17), applied electrical stimuli to isolated frog gastrocnemius muscle and obtained similar results; an increase in stimulation rate from 10 shocks per minute to 60 shocks per minute for six minutes produced a nine-fold increase in rate of glycogenolysis. From these findings, it might be hypothesized that phosphorylase a levels should increase in proportion to the rate of stimulation or muscle contraction, and that the high glycogen levels, still present at fatigue, are a reflection of the inability of the enzyme to degrade the substrate possibly as a consequence of reconversion to form b. In a theory proposed by Cori (17), phosphorylase a levels are said to rise during each contraction and fall back toward the baseline, by reconversion, during each pause. If stimuli were continued, the baseline could gradually rise to produce an elevated measure for phosphorylase a. When muscle fatigue has set in, the baseline would drop back below initial levels as a result of some stimulus to reconversion.

Evaluation of this hypothesis is difficult because experimental evidence and techniques are lacking. Several researchers have presented evidence obtained by varying electrical stimulation rates, but no studies have examined phosphorylase changes in response to maximal voluntary exercise.

Effects of Training Upon Phosphorylase Activity

Training and high levels of fitness, have been found, in both animals (48, 64) and man (2, 3, 49, 70) to be associated with high muscle glycogen concentrations. The glycogen, however, does not appear to be depleted to a lower baseline in the trained than in the untrained group (49), when relative work loads are used. No studies have actually compared the slope of the glycogen depletion curve, in trained and untrained subjects, with a view to determining the significance of the difference. Further research is needed to fully elucidate the effects of training upon glycogen breakdown rate.

Only two (29, 83) quantitative studies were found which related phosphorylase activity to training. Gould and Rawlinson (29) studied the effects of swim training, 30 minutes per day for six weeks upon the levels of total phosphorylase in resting skeletal muscle of rats. No significant difference was obtained between the trained and untrained groups. Holloszy (36), however, has suggested that the apparent absence of enzyme biosynthesis in this study was a manifestation of the mild training program. Holloszy (36) has reported that even untrained rats in his laboratory were capable of swimming for six hours. Studies of the effects of similar mild training programs on other enzymes, have produced similar results (31).

Yampolskaya and Yakovlev (82, 83) measured the "phosphorylic activity" displayed by resting muscle from

trained and untrained rats. The hexose phosphate produced from glycogen was significantly higher in the trained than in the untrained animals even though training was very mild. Gould and Rawlinson (29), however, state that these results were probably a reflection of kinetic responses, since long-term incubation procedures were used for assay of large samples of tissue brei.

Despite the dearth of information regarding training adaptations of the phosphorylase enzyme, the respones of other enzyme systems to various physiological stimuli (27, 32, 36, 43, 48) suggest that such adaptations are probable. Some encouraging findings (37, 71) in this regard are that detraining, as produced by denervation or immobilization, has resulted in significantly lower levels of phosphorylase in muscle. Muscle glycogen levels have also been reduced by detraining (51).

CHAPTER III

METHODS AND PROCEDURES

Fifteen male subjects 17 to 28 years of age participated in the study. Eight of these were members of the University of Alberta wrestling or track team and underwent a four month program of rigorous training and competition. The other seven subjects seldom engaged in exhaustive exercise and were classified as the control group. Only one series of tests was conducted on the control group but the active group was tested both pre- and post-training.

Each series of tests consisted of three sessions on a bicycle ergometer: a preliminary work capacity determination, followed by both maximal and submaximal exercises to exhaustion. During the maximal and submaximal tests, muscle samples were taken. These samples were weighed and frozen and at a later date analyzed for phosphorylase enzyme activity.

Work Capacity Determinations

The MVO_2 of each subject was initially assessed, on a previously calibrated Monark bicycle ergometer, using the Astrand bicycle ergometer test (6, 7), as detailed by Glassford *et al.* (26). The MVO_2 of the members of the active group was reassessed prior to the post-training tests.

In the Astrand test the subject pedalled at 50 r.p.m.

for six minutes at each work load. A metronome was used to control the rate of pedalling, but the actual number of revolutions was not measured. The starting work load was 600 kpm and work loads were raised at the discretion of the examiners, by increments of 300 kpm at low work levels and 150 kpm at the higher work levels. Five minutes rest was allowed between successive increments. An expired air sample was collected for one minute between the fifth and sixth minutes of exercise at each work load.

The criterion for termination of the test was an increase of 80 ml or less in oxygen uptake at successive work loads (5). The larger of the final two oxygen uptake values was recorded as the MVO_2. The penultimate work load was recorded as the maximal work load. The work load corresponding to an oxygen uptake of 70% MVO_2 was found by interpolation and recorded as the submaximal (70%) work load.

The Maximal and Submaximal Tests

The work loads for the submaximal and maximal tests were those found by the Astrand test to elicit oxygen uptakes of 70% and 100% of the MVO_2, respectively. These work loads were maintained throughout exercise and both tests were continued to exhaustion. The maximal test thus required a brief intensive effort, while the submaximal test was a prolonged exercise session. The criterion for termination of the exercise was the inability of the subject

to maintain the pedal rate of 50 rpm. The duration of the exercise was recorded in each case. With few exceptions, the submaximal test was performed first and several days later the maximal test was administered.

A muscle biopsy needle of the type described by Bergstrom (11) was used to take samples from the vastus lateralis portion of the quadriceps. In the maximal test only three biopsies were taken: one at rest before the exercise, one at the time of declared fatigue, and one after ten minutes of recovery. During the submaximal test, samples were taken before the exercise, every 20 minutes during the exercise, at the time of fatigue, and after ten minutes of recovery. The initial and recovery biopsies were taken with the subject in the reclining position; the remainder were taken while the subject was still sitting on the bicycle. At no time during the exercise did the subject cease to pedal for longer than the few seconds needed to inject the anaesthetic or to insert and extract the biopsy needle.

Controls

No rigid controls were enforced but the subjects were asked to refrain from smoking, alcohol, caffeine, fasting, carbohydrate loading of the diet, and exhaustive exercise for 24 hours prior to testing, since each of these factors has been implicated in some way with metabolic balance, glycogen storage, or enzyme activity (2, 3, 18, 53, 70). The subjects exercised in shorts, with T-shirt

and running shoes optional. Water was made available during the tests if requested.

Muscle Biopsy Technique

In preparation for muscle sampling, a section of the skin overlying the vastus lateralis of the left or right leg was shaved and made aseptic. Ten millilitres of two percent xylocaine (without epinephrine) were injected to produce local anaesthesia and a scalpel was used to make a small incision in the skin and fascia of the muscle. Through this incision, the biopsy needle could be inserted at will and a small sample of muscle extracted. All the samples required during any one test were taken through the same incision. The biopsy needle was usually advanced three to five centimetres into the body of the muscle, but no attempt was made to control the precise depth of the insertion. During prolonged tests an additional dose of xylocaine was injected every 40 minutes.

The muscle biopsy needle was composed of three cylindrical metal tubes which slid inside one another (Figure 7). The hollow outer needle (3.5 to 5 mm in diameter) converged to a sharp point and had a small window near the tip. A hollow cylinder with a plunger at one end and a sharp cutting edge at the other, fitted neatly inside the outer needle and, when depressed, sliced off the small piece of muscle which bulged through the window. The needle was then extracted from the muscle and the solid innermost cylinder,

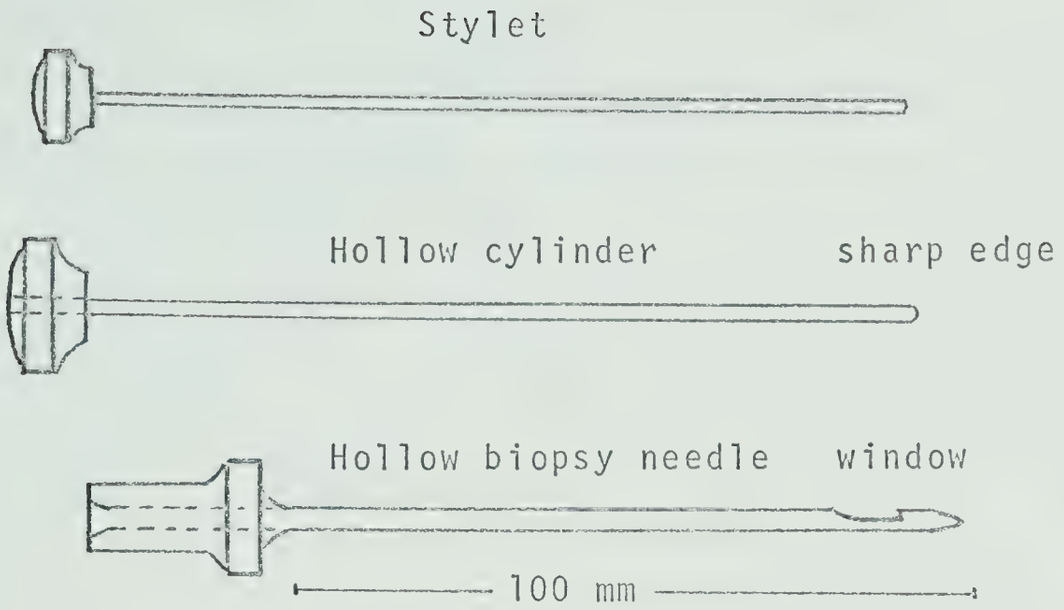


FIGURE 7

PUNCH BIOPSY NEEDLE FOR MUSCLE BIOPSY
The stylet was used for pushing out biopsy specimens from the cylinder. (From Bergstrom, 1962 (11)).

the stylet, was used to remove the sample.

Weighing and Freezing of the Sample

After extraction from the body, the muscle sample was transferred to the pan of a Roller-Smith Precision Balance. All visible fat and connective tissue was removed with a probe and forceps, and the sample was weighed. The weight was recorded, and the sample was transferred to the

bottom of a test tube. The test tube was then capped and immediately placed in a mixture of dry ice and 95 percent ethyl alcohol to freeze the sample and to prevent, as much as possible, further enzyme conversion. The total time taken, from the moment of extraction until freezing, did not exceed two minutes. The weight of any excess blood remaining in the pan was subtracted to obtain the weight of the sample in the test tube. At the end of each testing session all of the frozen muscle samples were taken to the Surgical-Medical Research Institute Laboratory at the University of Alberta and stored in a deep freeze (-60°F) to await biochemical analysis.

Phosphorylase Assay

Phosphorylase activity was assessed by the isotopic technique of Russell, Tougas and Taylor (69). This method was developed especially for the rapid assay of glycogen cycle enzymes in small samples of muscle such as those obtained by muscle biopsy. Muscle samples weighing 25 to 75 mg were suitable for assay. Phosphorylase determinations were made in the reverse direction, using G-1-P-^{14}C as the substrate for reaction with muscle homogenates, and the rate of incorporation of labelled glucose into primer glycogen was measured with a liquid scintillation counter.

Glycogen (Fisher Scientific Co., Pittsburgh, Pa. 15219) was purified by chromatography on Bio Rad AD-1 anion exchange resin to remove AMP. All other chemicals used

were reagent grade. Water distilled in Pyrex was used throughout.

The tissue samples were frozen with liquid nitrogen in 4 ml of buffer solution containing 20 mmol/l sodium glycerophosphate, 1 mmol/l EDTA, 100 mmol/l NaF, and 20 mmol/l mercaptoethanol, pH 6.10 $\pm$ 0.05*. The frozen buffer and sample were ground to a fine paste in liquid nitrogen with a mortar and pestle, and then allowed to thaw. The resulting suspension was centrifuged for 5 min at 1500 g and the supernatant fluid was used for the assay. The extracts were kept on ice at all times. The activity was stable for several hours, but decreased drastically if the sample was kept overnight at 0°C. The homogenates fractionated were centrifuged for 30 min at various speeds in a Type 40 rotor in an L3-50 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif. 94304).

For the assay procedure, tubes were prepared containing 0.1 ml of the substrate solutions. These were equilibrated at 30°C for 15 min in a water bath, together with the muscle extract samples. Samples of 0.1 ml were then

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* NaF and EDTA were present to inhibit the actions of phosphorylase phosphatase and phosphorylase-b-kinase respectively, and thus prevent further interconversion between the phosphorylase a and b forms (19).
The glycerol served to maintain the solution in liquid form at very low temperatures, and thus allowed the inhibitors to penetrate the muscle powder at these low temperatures (19).
Mercaptoethanol was included to stabilize the phosphorylase at the pH of the reaction.

added to the substrate tubes, shaken, and timed. Reaction was allowed to proceed for 5 min for the phosphorylase measurements. The reactions were stopped by adding 1 ml of an ice-cold solution containing trichloroacetic acid (6 g/100 ml), 2 mg of LiBr, and 1 mg of glycogen. The proteins were removed by centrifuging at 1000 g for 1 min, and the supernatant fluid was decanted. Cold 95% ethanol, 2 ml, was added and the samples were allowed to set for 20 min to precipitate the glycogen, then centrifuged at 2000 g for 10 min, the supernatant fluids decanted and discarded. The precipitates were washed with 66% ethanol and centrifuged two more times. The glycogen pellets were finally resuspended in 1 ml of ethanol and washed into counting vials with 1 ml of water. The vials were taken to dryness in a vacuum oven at approximately 40°C. One ml of "NCS" reagent, a basic solubilizing agent (Nuclear-Chicago Corp., Des Plaines, Ill. 60018), was added and the vial well shaken. Fifteen ml of toluene containing 4 g of PPO (Beckman Instruments, Inc.) and 50 mg of POPOP (Beckman Instruments, Inc.) per litre were added and the samples were counted in a liquid scintillation counter (Nuclear Chicago Mk 1). Counting efficiencies were determined by the channels-ratio method. Blank samples were run, the sample being added after the quenching solution of trichloroacetic acid.

The compositions of the substrates were:

Solution (a): 2 g of glycogen per 100 ml, 32 mmol/l of G-1-P-^{14}C (4.3×10^7 dpm/mmol) (New England

Nuclear Corp. and Sigma Chem. Co.) in the buffer system used for homogenization, pH 6.1.

Solution (b): as for solution (a), plus 2 mmol/l of AMP (Sigma Chemical Co.).

The substrate solutions were stored frozen in individual tubes, and remained usable for at least one month. Substrate solution (a) was used for determining phosphorylase a activity; substrate solution (b), which contained the AMP, was used to obtain the total phosphorylase (a plus b) activity. Phosphorylase b activity was then calculated by subtraction.

Statistical Analysis*

Two analysis of variance designs were applied to the data. Design I (81:302-319) was used to compare the control group with the experimental group (pre-training) and so had repeated measures on one factor only. Design II (81:319-337) compared the same group of subjects pre- and post-training and so had repeated measures on both factors. Six separate analyses were performed, using each design, to investigate the effects of maximal and submaximal exercise and training upon the activity levels of phosphorylase a, phosphorylase b and total phosphorylase. Only the initial, final, and recovery values were used in these analyses.

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* All statistical procedures employed are outlined in detail in Appendix C.

The values obtained at 20 minute intervals, during submaximal exercise, were converted to percent values by linear interpolation and treated only graphically.

In addition to the analysis of variance tests, certain multiple comparisons, selected a priori, were made between cell means using Tukey's w (HSD) procedure (77). Initial, final and recovery means, and also corresponding cell means for the two groups of subjects, were compared in this way.

The analyses for designs I and II were not statistically independent, since the pre-training results for the experimental group were used in both designs. This would normally necessitate the use of a more rigorous alpha level. Compensations were made, however, by the application of the Greenhouse and Geisser conservative test (30, 81:305) and by the use of a stringent multiple comparison procedure, Tukey's w (77, 81:88). Consequently, the 0.05 level of alpha was still considered as the point of significance for all F ratios and multiple comparisons.

CHAPTER IV

RESULTS

Reactions of Subjects to Muscle Sampling

Subjects seldom complained of anything more than mild pain and discomfort, during or after the test. Most subjects reported almost complete healing within a few days, although in one case a mild haematoma did develop. Bleeding was not excessive and the incision in the working muscle did not appear to interfere with exercise performance.

Phosphorylase Activities

Tables 1 to 3, Figures 8 to 13 and Appendices B and D display the results of the study. Appendix B contains the raw data, while Appendix D presents the summary tables of cell means, respectively. Table 1 collates the subject particulars and work capacities for the various groups, while Tables 2 and 3 summarize the significant results from analysis of variance and Tukey's w procedures. Figures 8 to 13 are graphical representations of the means contained in Appendix D for phosphorylase a, phosphorylase b, total phsophorylase, and phosphorylase a as a percentage of the total enzyme activity.

Comparison of Control Group with Pre-Training Group

Maximal and submaximal work loads and work times and

the MVO_2 per kilogram body weight were similar for the control and pre-training groups (Table 1). Analysis of variance, however, revealed significant Groups and Treatments main effects, but only for phosphorylase a content of muscle during submaximal exercise (Table 2). The interaction effect from this analysis was not significant, indicating that the trends in the a levels, from initial to final recovery samples, were the same for the two groups. No comparisons were made between pairs of cell means.

Analysis of Variance Applied to Pre- and Post-Training Groups

The analysis of variance procedures, when applied to the data for the experimental group pre- and post-training, yielded significant F ratios for the Groups main effects only (Table 2). In all cases, except phosphorylase b levels during maximal exercise, the pre-training phosphorylase levels were significantly different from the post-training results ($P < 0.05$). The Treatments main effect was not significant under any of the analyses. The Groups by Treatments interaction effect was also not significant throughout, indicating that the trend in phosphorylase activity was the same throughout rest, fatigue, and recovery, in the two groups.

Effects of Exercise Upon Total Phosphorylase

Short-term maximal exercise was found to produce little change in total phosphorylase content (Figure 9).

TABLE 1

MEAN WORK LOADS AND WORK CAPACITIES

Group	n	Age (Range)	MVO_2/kg (ml/min/kg)	Submaximal Work Load (kpm)	Submaximal Work Time (min)	Maximal Work Load (kpm)	Maximal Work Time (sec)
Sedentary		18 to 26	44.01	1007	74	1496	532
Pre-training		17 to 28	43.73	1126	83	1611	579
Post-training		17 to 28	55.98	1269	68	1710	551

TABLE 2

SUMMARY OF SIGNIFICANT RESULTS FROM

ANALYSIS OF VARIANCE

Design I. Control and Pre-Training Groups

Source of Variation	Submaximal			Maximal		
	a	b	Total	a	b	Total
Groups (Control vs. Pre-train.)	**					
Treatments (I. vs. F vs. R)		*				
Interaction						

Design II. Pre- and Post-Training Groups

Source of Variation	Submaximal			Maximal		
	a	b	Total	a	b	Total
Groups (Pre vs. Post)	*	*	*	**		**
Treatments (I vs. F vs. R)						
Interaction						

* $P < .05$
** $P < .01$

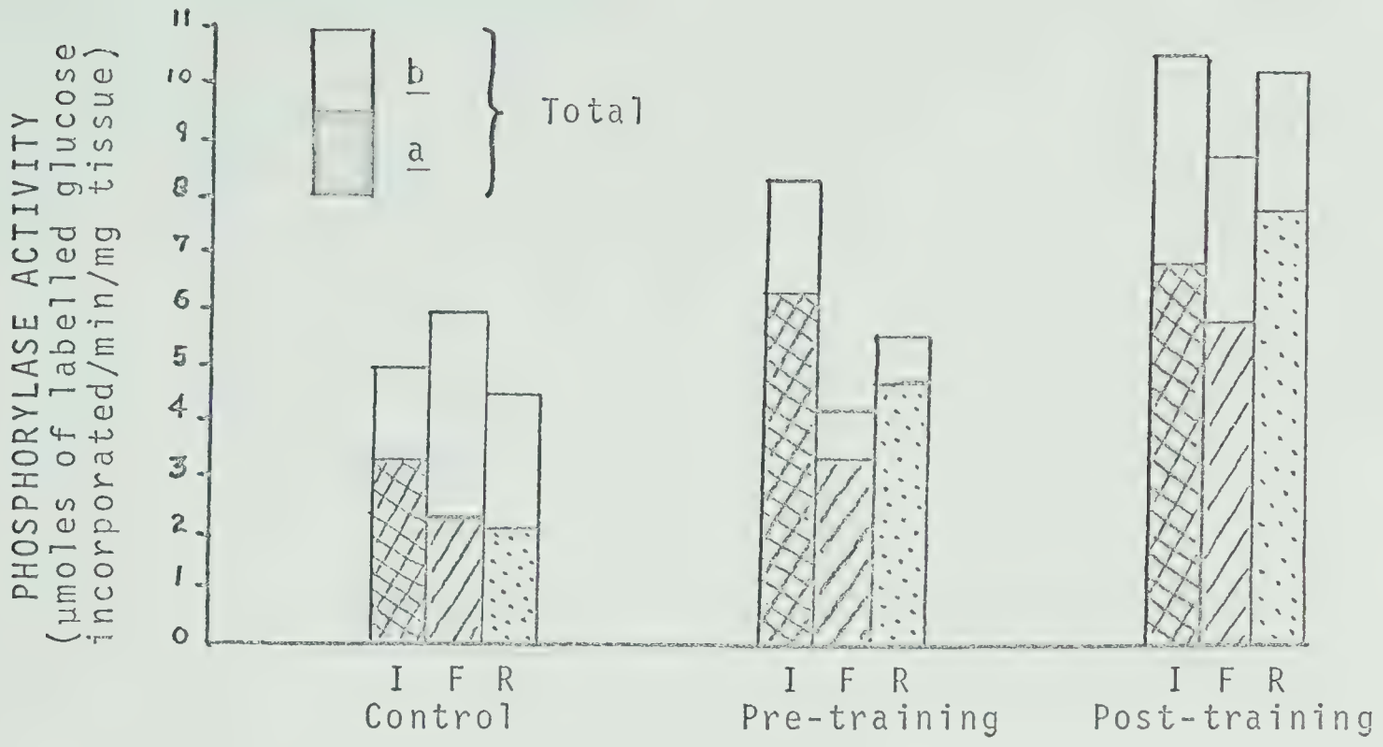

FIGURE 8

PHOSPHORYLASE ACTIVITY DURING SUBMAXIMAL EXERCISE

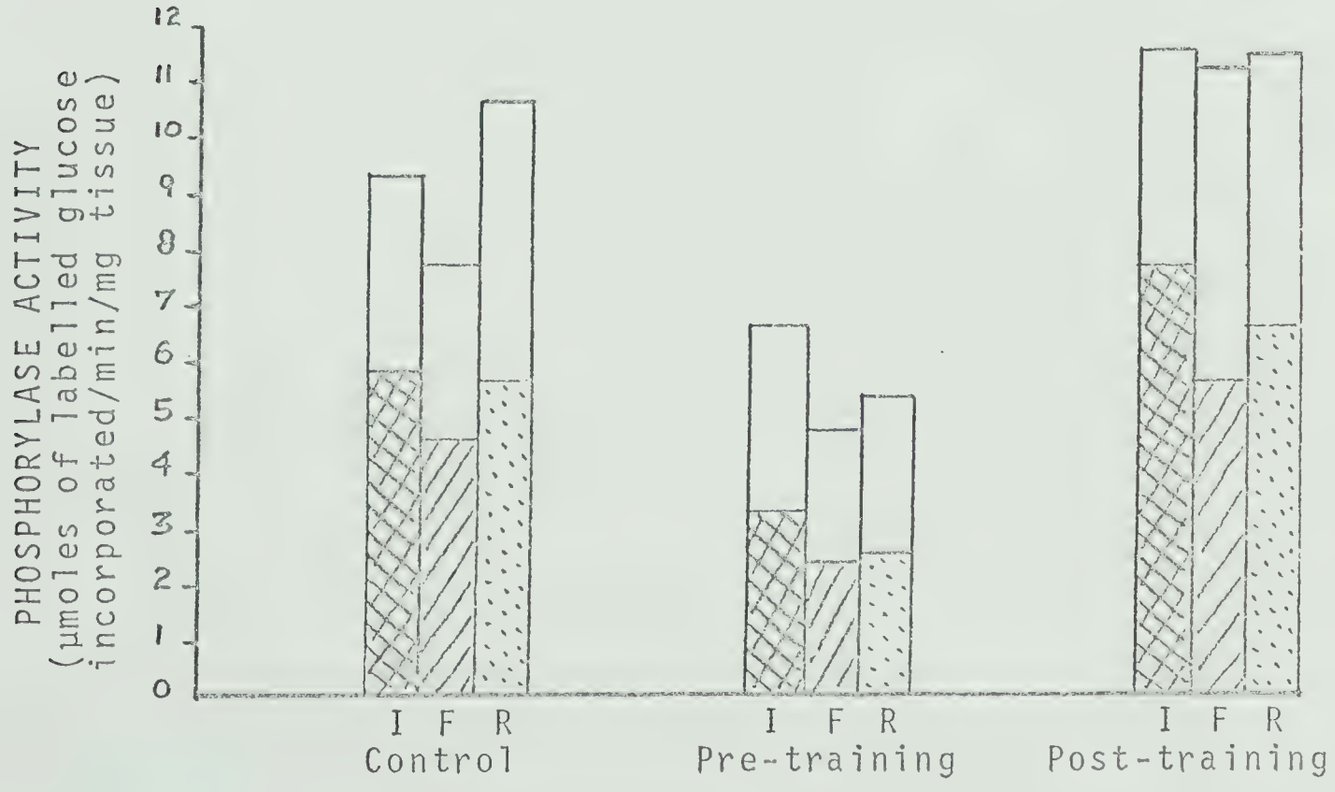

FIGURE 9

PHOSPHORYLASE ACTIVITY DURING MAXIMAL EXERCISE

Initial, fatigue and recovery values were not significantly different from each other, in either the pre-training or post-training groups (Table 3). With prolonged submaximal exercise, however, a trend could be detected in the results. Figure 10 (bottom) shows the curves of best-fit to the plotted means for the three groups during submaximal exercise. A gradual decrease in total phosphorylase content of the muscle was evident in the experimental group tested both pre- and post-training,* and yet the initial, fatigue and recovery values were not significantly different (Tables 2 and 3 and Figure 8).

Effects of Submaximal Exercise Upon Phosphorylase a Levels

The best-fit curves in Figure 10 (top) suggest that a slight decrease in phosphorylase a levels might have occurred throughout submaximal exercise. In each group, the mean a levels were lower at fatigue than initially (Figure 8), but in no case was the difference significant (Tables 2 and 3). Phosphorylase b content was remarkably stable throughout the exercise (middle frame of Figure 10). The proportion of the total enzyme present in the a form was considerably variable (Figure 11).

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* A trend analysis could not legitimately be applied to these results, since many of the original 25, 50 and 75 percent values were derived by linear interpolation from obtained values; this procedure would tend to minimize the deviation from the best-fit curve and so would yield a greater chance of significance.

TABLE 3

SIGNIFICANCE OF MULTIPLE COMPARISONS

USING TUKEY'S w PROCEDURE

Pre- and Post-Training Groups[X]

Comparison[+]	Submaximal			Maximal		
	a	b	Total	a	b	Total
$\bar{I} - \bar{F}$						
$\bar{I} - \bar{R}$						
$\bar{F} - \bar{R}$						
$I_1 - F_1$						
$I_1 - R_1$						
$F_1 - R_1$						
$I_2 - F_2$						
$I_2 - R_2$						
$F_2 - R_2$						
$I_1 - I_2$				*		
$F_2 - F_2$						*
$R_1 - R_2$				*		*

X No multiple comparisons were performed on the data for control and pre-training groups.

+ Subscript 1 refers to pre-training group
Subscript 2 refers to post-training group
I = initial; F = final; R = recovery; $\bar{I}$ = $(I_1 + I_2) \div 2$, etc.

* $P < .05$

** $P < .01$

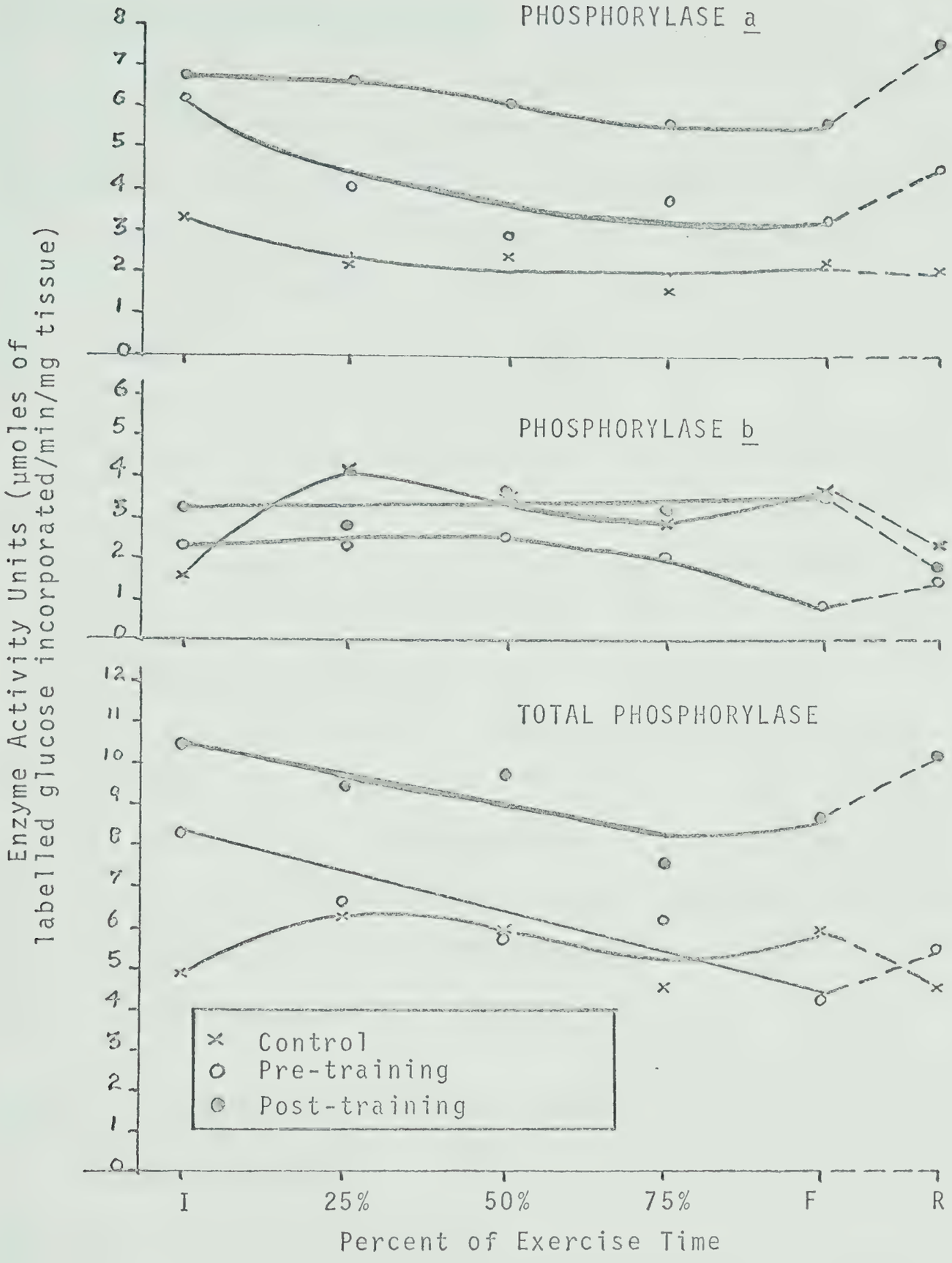

FIGURE 10

TRENDS IN PHOSPHORYLASE ACTIVITY DURING SUBMAXIMAL EXERCISE

The 25%, 50% and 75% points were, in many cases, derived by linear interpolation from obtained values.

Effects of Maximal Exercise Upon Phosphorylase a Levels

The results obtained in response to maximal exercise were very similar to the submaximal exercise results. Again no significant differences were found between initial, final and recovery values within any one group (Tables 2 and 3). The levels of phosphorylase a and phosphorylase b and the proportions of the two forms of the enzyme, were remarkably constant in the samples (Figures 9, 12).

The control group phosphorylase levels for maximal exercise appeared considerably high when compared to the pre-training group (Figure 9). The differences were significant for phosphorylase a and large, though not significant for total phosphorylase (Table 2). The control group values were also considerably higher than the values for the same subjects during submaximal exercise (Figures 8,9). These findings suggest that the submaximal exercise produced some form of stress, in the sedentary control subjects, which was still reflected in high phosphorylase levels at the time of maximal exercise, approximately one week later.

Effects of Training Upon Total Phosphorylase

Analysis of variance yielded significant F ratios for the Groups main effect for both maximal and submaximal exercise (Table 2), indicating that some adaptation to training had occurred.

The initial, final, and recovery levels of total phosphorylase, were always higher in the post-training subjects

than in the same subjects pre-training, for both maximal (Figure 9) and submaximal (Figure 8, 10) exercise, but only a few of these differences proved significant using Tukey's test (Table 3). The initial levels of total enzyme were not significantly higher in the post-training than in the pre-training subjects. The final values and the recovery values were also not different after the submaximal exercise. In response to maximal exercise, however, the fatigue and recovery values were both significantly higher in the trained subjects.

Effects of Training Upon Levels of Phosphorylase a

The pre-training levels of phosphorylase a were always lower (Figure 8, 9) than the corresponding post-training levels, but again only two differences were significant: the initial and recovery values of maximal exercise. The differences in the a levels appeared to be annulled, however, when phosphorylase a was expressed as a percentage of the total activity (Figures 11, 12).

Glycogen Breakdown During Maximal Exercise

Figure 13 indicates that more rapid glycogen breakdown occurred in the post-training than in the pre-training subjects. The post-training group had higher initial levels of glycogen and used 0.41 g/100 g tissue, compared to 0.32 g/100 g in the pre-training group.

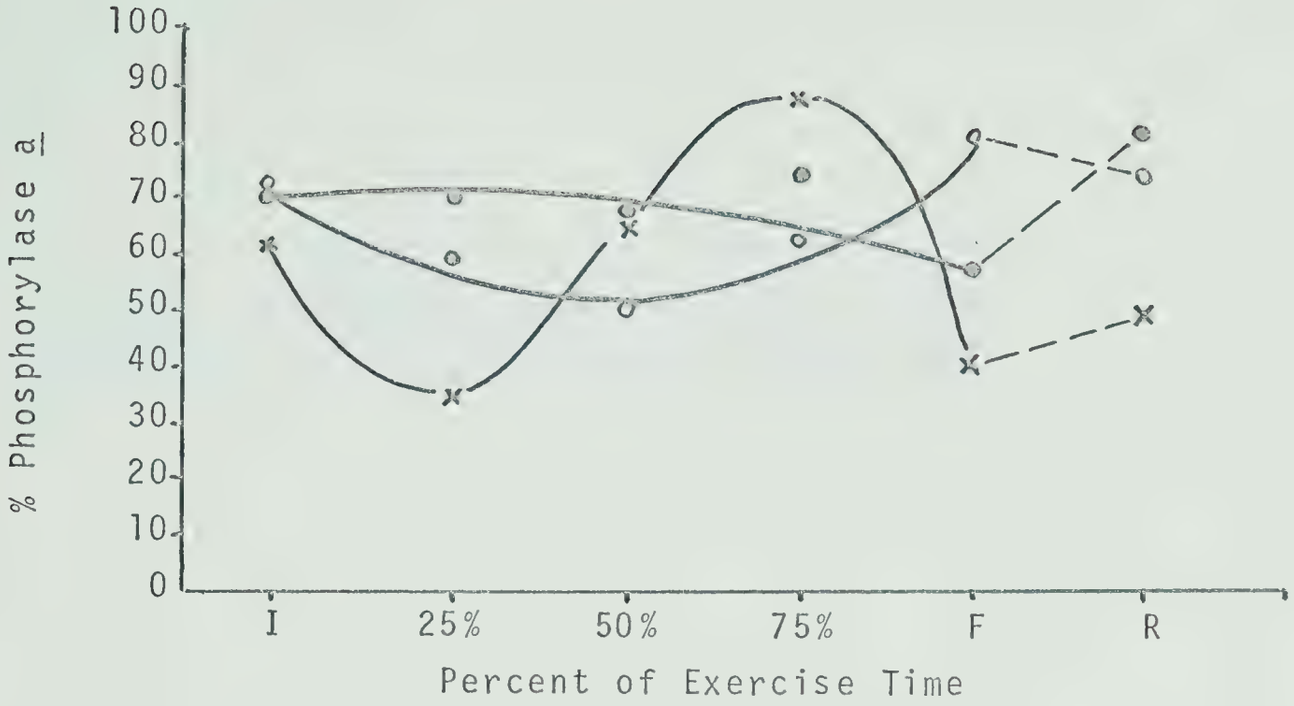

FIGURE 11

CHANGES IN PHOSPHORYLASE a AS PERCENTAGE OF TOTAL PHOSPHORYLASE DURING SUBMAXIMAL EXERCISE

x = control o = pre-training ⊙ = post-training

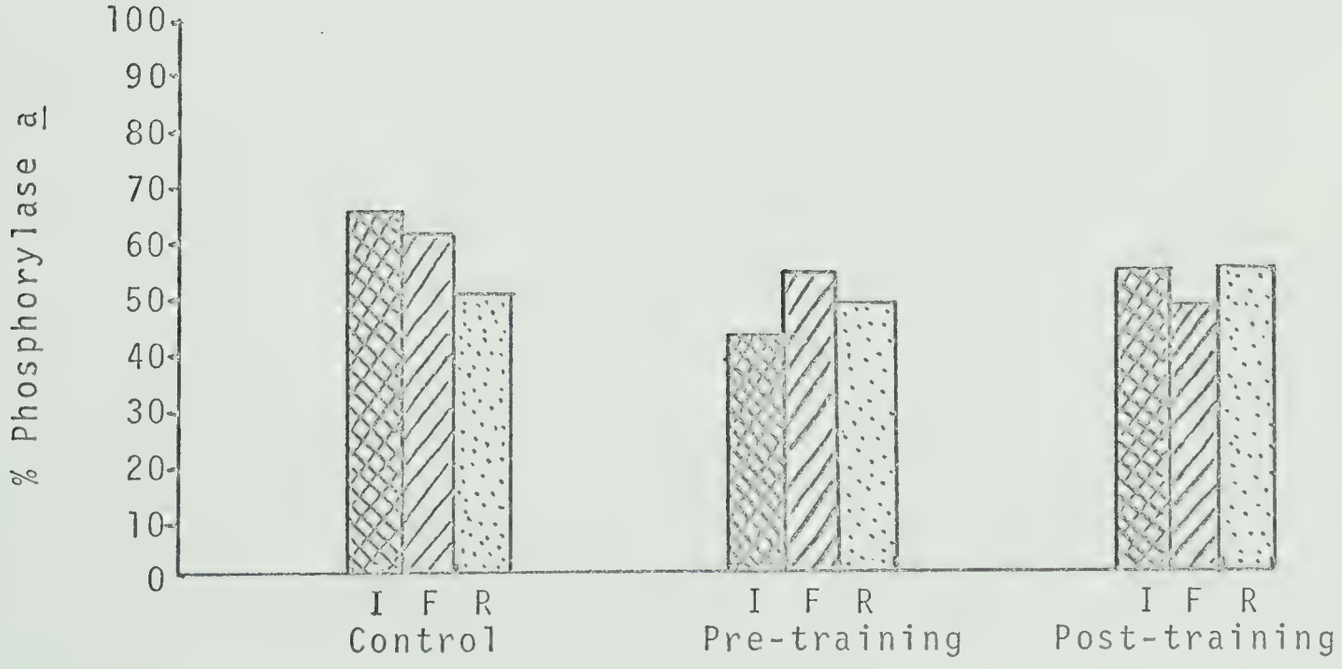

FIGURE 12

CHANGES IN PHOSPHORYLASE a AS PERCENTAGE OF TOTAL PHOSPHORYLASE DURING MAXIMAL EXERCISE

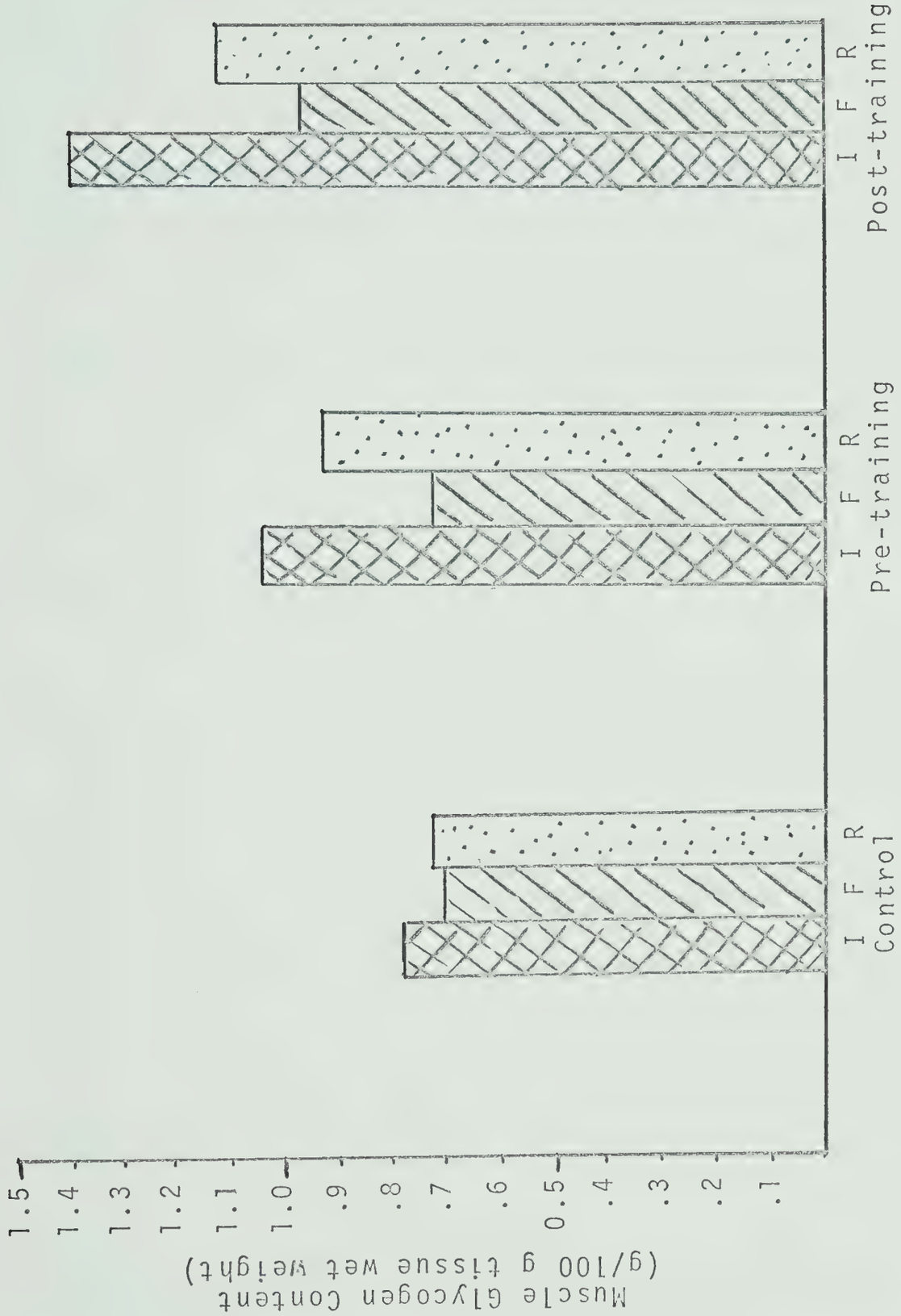

FIGURE 13

EFFECTS OF MAXIMAL EXERCISE UPON MUSCLE GLYCOGEN CONTENT

CHAPTER V

DISCUSSION

The analysis of variance performed on the data for control and pre-training groups produced a significant F ratio for the Groups main effect, with regard to phosphorylase *a* levels. Consequently, generalizations can not legitimately be made to other populations of semi-active subjects. No multiple comparisons were performed on these results. Moreover, practical considerations did not permit a second testing of the control group, coincident with the post-training tests on the experimental group.

Any differences detected, then, between the pre-training and post-training subjects can not be strictly attributed to the effects of the training program; other, extraneous, factors could have been operating. In addition, the control group means for total and % *a* levels were subject to considerable variation and fluctuation, especially during submaximal exercise. In view of these results, discussion will be limited to comparisons between the pre-training and post-training subjects.

Effects of Submaximal Exercise Upon the Phosphorylase *b* $\rightleftarrows$ *a* Conversion

Comparisons of the initial, final and recovery values recorded during prolonged submaximal exercise at 70 percent

MVO_2, revealed no significant changes in total, *a* or percent *a* levels of the phosphorylase enzyme. These results, which are in accord with those of Canal and Frattola (13) induced by six hours of swimming in rats, would tend to imply that the *b* to *a* conversion mechanism is not important in exercise of this kind; either energy from skeletal muscle glycogen is not required at a rate sufficient to merit conversion to the active form or else breakdown can occur rapidly in the absence of phosphorylase *a*. Other studies (2, 3, 49, 70) have found only gradual decline in muscle glycogen stores during submaximal exercise in man, so the former would seem to be the more plausible explanation.

In the present study, phosphorylase *a* was actually quite high initially (65 to 80 percent of the total recorded activity) and showed a slight but progressive decrease in absolute levels throughout the exercise (Figure 10, top panel). Similar high values were found by Canal and Frattola (13). The final phosphorylase *a* levels, however, were not significantly different from the initial values in either the pre- or post-training subjects. Partly, the high *a* levels, both initially and throughout exercise could be attributed to an involuntary contractile response of the muscle to rapid freezing. Cori (17) found that total phosphorylase was not changed by the freezing process, but that the amount of enzyme in the *a* form showed a rise of 15 percentile points. Even when the effects of freezing are subtracted, however, the phosphorylase *a* levels still appear high in comparison

to results obtained with small animals (17, 18, 19). If high phosphorylase *a* concentrations are normal in resting human subjects, then the normal glycogen turnover would have to occur in the face of these high concentrations of active enzyme. Such rapid turnover would not seem probable from an efficiency viewpoint. More probably the high values are a response to handling or treatment of the samples.

The observation that the amount of active phosphorylase remains little changed, from resting values, during such prolonged exercise, provides support for the theory of glycogen as a reserve energy supply. In submaximal exercise, when the body is able to achieve and maintain, a steady metabolic state, rapid glycogen breakdown should not be required. The gradual glycogen store depletion that has been frequently observed (2, 3, 49, 70) could be the result of kinetic factors operating either to render phosphorylase *b* partially active or to inhibit the synthetase enzyme system.

An alternative explanation could view the recorded initial values for phosphorylase *a* as falsely high, so that the *a* levels actually increase at the start of exercise. The high initial *a* levels could, under this theory, be a manifestation of stress and catecholamine secretion in the apprehensive subjects. The cyclic-AMP acceleration of P-*b*-Kase activation has been well established (45, 46, 54, 63), and can be initiated by epinephrine (10, 45, 63).

It is interesting that, although the absolute levels of phosphorylase *a* in the muscle showed a progressive, but

insignificant decrease, until fatigue, the percentage of total activity attributable to phosphorylase *a* remained quite stable. This was due to an accompanying declining trend in total phosphorylase. The decrease in total phosphorylase, though insignificant, certainly argues against the possibility of adaptive enzyme biosynthesis in the course of exhaustive exercise of 65 to 80 minutes duration. In the rat study by Canal and Frattola (13) the total phosphorylase activity was found to increase, but not significantly, over a six hour swim period.

The observations regarding the relative proportions of the *a* and *b* forms of phosphorylase should, however, be regarded with some reserve. A wide individual variation was observed (Appendix B). Considerable doubt must exist as to whether the observed *a* levels actually reflect those existing in the muscle. Cori (17) found that the percentage of phosphorylase *a* changed considerably in muscles of rats and frogs during three minutes immediately following tetanus. In fact the % *a* levels of rat gastrocnemius were found to be lower than in the controls after three minutes of recovery from tetanus. Danforth, Helmreich and Cori (19) substantiated these findings but also noted that the time taken for *a* levels to return to the baseline during recovery was inversely proportional to the stimulation frequency. The reconversion rate, during maximal or submaximal exercise of the kind used in this study, is not known, but some reconversion could be expected to occur in the 30 to 90 seconds bet-

ween the time of extraction and freezing of the sample.

Effects of Maximal Exercise Upon the Phosphorylase b $\rightleftarrows$ a Conversion

Comparisons of the initial, final and recovery samples, in response to maximal exercise which produced exhaustion in nine to ten minutes, revealed no significant changes in total, a, or % a levels of the phosphorylase enzyme. The results then, are very similar to those obtained with submaximal exercise. The percentage of phosphorylase in the a form was again quite high (40 to 60%) in initial samples and similar conclusions could be drawn. The total concentration of phosphorylase in the muscle was approximately the same before maximal as before submaximal exercise, within any one group of subjects, the control group excepted.

Despite the absence of change in the phosphorylase a levels during exercise, glycogen breakdown was still observed. These results again suggest that either the obtained initial a levels were falsely high or that kinetic factors were operating to increase the activity of phosphorylase b or inhibit synthetase enzyme.

Effects of Training Upon Total Phosphorylase

The analyses of variance of the total phosphorylase data indicated significant differences (Table 2) between the pre-training and post-training groups in both maximal ($P < .01$) and submaximal ($P < .05$) exercise. Throughout both types

of exercise, the total enzyme concentration was higher after the subjects had completed four months of rigourous training. These results imply that the concentration of the specific phosphorylase protein in muscle had actually been raised to higher levels by biosynthesis in response to training. The demonstration of such phosphorylase adaptation is in keeping with research findings from other enzyme systems in response to various physiological stimuli.

Tukey's HSD procedure was used to test the significance of the difference between corresponding cell means for the pre- and post-training subjects. During submaximal exercise, no pairs of means were significantly different, even though the post-training group were considerably higher in total phosphorylase activity of all times. With maximal exercise, the final values for the two groups and the recovery values for the two groups were significantly different ($P < .05$); the difference in the two initial values was large but not significant. The failure of several of these differences to attain significance was probably a consequence partly of the stringency of the Tukey's test, but also of the large error terms involved in the assessments. These large error variances, in turn, were probably a reflection of inadequate experimental controls and techniques.

Effects of Training Upon the Phosphorylase b $\rightleftarrows$ a Conversion

The analyses of variance of the phosphorylase a data indicated significant differences (Table 2) between the pre-

training and post-training groups in both maximal ($P < .05$) and submaximal exercise. However, when the *a* content was considered in relation to the total phosphorylase concentration, and expressed as % *a*, no differences were apparent between the two groups. The higher absolute levels of phosphorylase *a* in the post-training group, were significant only at the initial and recovery stages of maximal exercise, even though other differences were large. These higher *a* levels in the post-training subjects could undoubtedly account for the more rapid glycogen breakdown rate observed in response to training.

CHAPTER VI

SUMMARY AND CONCLUSION

Summary

Human subjects were exercised on a bicycle ergometer, both pre- and post-training, at work loads designed to induce 70% and 100% MVO_2. A control group was used for comparisons with the pre-training group. A biopsy needle was used to take initial, fatigue and recovery samples from the vastus lateralis muscle of the thigh and these samples were assayed for changes in glycogen content and phosphorylase enzyme activity.

Neither maximal nor submaximal exercise was found to produce any significant change in total phosphorylase or phosphorylase *a* activity in the muscle. Training, however, resulted in high total phosphorylase and phosphorylase *a* concentrations at all times throughout exercise. The increases observed in total enzyme strongly implied adaptive enzyme biosynthesis in the muscle in response to training, although most differences between pairs of means failed to attain statistical significance under Tukey's stringent HSD test. The increases in phosphorylase *a* concentrations with training were also not statistically significant in many cases, especially with submaximal exercise, but the size of the differences observed could probably account for the more rapid glycogneolysis in trained subjects. The percentage of the

enzyme in the a form was similar in pre- and post-training subjects at the relative work loads employed.

Because of the sensitivity of the phosphorylase interconversion mechanism to freezing and to time taken to weigh the sample, the recorded levels of phosphorylase a and b cannot be considered reliable or valid indices of the proportions actually existent in the body. Total phosphorylase measurements are not, however, subject to these effects. The inability to exert adequate supervisory control over the behaviour of human subjects, could also be held responsible for some of the large individual variations observed.

Conclusion

Within the delimitations of the study, the following conclusions were drawn:

(1) In both pre- and post-training groups, fatigued muscle was associated with lower levels of phosphorylase a than either the initial or recovery samples, but the differences between the three means were not significant for either maximal or submaximal exercise.

(2) Training produced an adaptative response in muscle, characterized by biosynthesis of the specific phosphorylase protein, but a similar change in total phosphorylase was not observed during single bouts of either short maximal or prolonged submaximal exercise.

(3) The experimental subjects were also found to have higher concentrations of active enzyme, phosphorylase a, at the

end of the training program, but the percentage of the enzyme in the a form was unchanged due to the proportional rise in total phosphorylase.

(4) The questionable validity of the phosphorylase a and b results did not permit conclusions to be drawn with respect to the relative importance of these two enzyme forms in glycogenolysis or the onset of fatigue.

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APPENDIX A

PERMISSION FORM, DATA SHEET AND DEFINITIONS

Definitions

Phosphorylase a activity is the rate at which the phosphorylase reaction proceeds in the absence of AMP. When activity is assessed in the laboratory using an optimum condition assay situation, activity is proportional to concentration.

Phosphorylase b activity is the additional rate at which the phosphorylase reaction proceeds in the presence of AMP activator. In an optimum condition assay situation, activity is proportional to concentration.

Total phosphorylase activity is the total rate at which the phosphorylase reaction proceeds in the presence of AMP. In an optimum condition assay situation, activity is proportional to concentration.

Phosphorylase activation refers to the conversion of phosphorylase enzyme from the inactive b form to the active a form.

Maximal exercise is such that the work load is intensive, oxygen uptake is maximal, and exhaustion is reached in a short period of time.

Submaximal exercise is such that the work load is moderately severe, oxygen uptake is below maximal, and the exhaustion point is reached only after a prolonged exercise session.

UNIVERSITY OF ALBERTA

FACULTY OF PHYSICAL EDUCATION

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

SUBJECT.....DATE.....TIME A.M. P.M.

1. I agree to participate in an investigation and in relation to this hereby authorize Drs. ..... and/or such assistants as may be selected by them, to perform the following procedure(s):

.....

.....

.....

2. Drs. ..... have explained the purpose of this study and I understand the routine of the procedure outlined above.

....
Witness Signature of Subject

If the subject is unable to sign or is under 21 years of age, complete the following:

The subject is a minor (..... years of age).

or

The subject is unable to sign because .....

As the closest relative or legal guardian I hereby sign on his/her behalf:

.....
Witness Signature Relationship

DATA RECORDING SHEET

Name: ..... Age: ..... Height: ..... Weight: ..... Group: .....

1. Åstrand Test for MVO_2

	Maximal	70%
Work Load (kpm)	600	
VO_2 (5th minute)		
Comments		

2. Muscle Biopsy Sampling

	Date and Time of Day	Time of Sampling (min)										Duration of Exercise	Comments on Diet, Activity etc.
		I	20	40	60	80	100	120	140	F	R		
Submaximal Test (70% VO_2)													
Maximal Test (100% VO_2)													

APPENDIX B

RAW DATA

TABLE 4

SUBJECT PARTICULARS: CONTROL GROUP

Subject	Age	Height (ft)	Weight (lb)	MVO_2/kg (ml/min/kg)	Submaximal Work Load (kpm)	Maximal Work Time (min)	Submaximal Work Load (kpm)	Maximal Work Time (min)
B.H.	22	5'8"	150	48.80	800	60	1320	370
B.K.	21	5'9"	175	46.00	1100	40	1350	540
G.R.	19	5'9"	160	44.78	900	80	1440	540
L.Do.	18	5'9"	165	48.51	1050	100	1560	565
L.Dv.	25	5'10"	185	42.91	1100	60	1440	660
G.V.N.	26	6'2"	185	41.12	1100	100	1800	420
L.W.	19	5'11"	179	35.96	1000	80	1560	600

TABLE 5

SUBJECT PARTICULARS: EXPERIMENTAL GROUP

Subject	Age	Height (ft)	Weight (lb)	MVO_2/kg (ml/min/kg)	Submaximal Work Load (kpm)	Maximal Work Time (min)	Submaximal Work Load (kpm)	Maximal Work Time (sec)
Pre-Training								
J.K.	17	5'11"	135	49.00	900	80	1320	450
D.C.	26	6'0"	221	27.94	1050	60	1560	660
J.S.	22	6'2"	165	41.43	1100	80	1560	720
D.I.	23	6'1"	170	41.50	1100	100	1620	300
G.W.	28	5'6"	142	52.48	1100	71	--	-
L.B.	21	6'3"	175	38.43	1200	100	1620	840
R.D.	24	5'8"	160	49.35	1260	115	1800	480
S.G.	26	5'7"	151	49.66	1300	56	1800	600
Post-Training								
J.K.	17	5'11"	135	-	--	--	--	-
D.C.	26	6'0"	210	42.01	1320	60	1680	880
J.S.	22	6'2"	160	56.51	1320	72	1680	480
D.I.	23	6'1"	172	51.29	1200	72	1620	280
G.W.	28	5'6"	139	66.30	1200	60	--	-
L.B.	21	6'3"	177	55.81	1320	93	1680	720
R.D.	24	5'8"	160	59.27	1260	60	1800	410
S.G.	26	5'7"	158	60.70	1260	60	1800	535

TABLE 6

PHOSPHORYLASE *a* ACTIVITY* DURING SUBMAXIMAL EXERCISE

		Subject	Initial	20 min	40 min	60 min	80 min	Final	Recovery
Control Group	1.	B.H.	2.71	4.63	1.00	--	--	2.97	1.99
	2.	B.K.	1.19	0.78	--	--	--	0.45	0.68
	3.	G.R.	0.77	0.52	0.76	1.96	--	1.37	1.12
	4.	L.Do.	3.65	4.04	2.71	1.85	0.35	4.16	1.01
	5.	L.Du.	5.32	1.29	1.55	--	--	1.35	1.82
	6.	G.V.N.	5.74	1.00	4.26	4.67	3.29	4.81	2.15
	7.	L.W.	3.75	1.44	4.26	1.47	--	0.74	2.77
Experimental Group Pre-Training	1.	J.K.	5.37	3.16	1.99	3.14	--	4.57	5.43
	2.	D.C.	2.95	2.51	3.71	--	--	3.47	5.86
	3.	J.S.	6.42	1.45	3.27	2.26	1.94	3.52	3.79
	4.	D.I.	9.93	4.49	4.68	2.88	7.62	5.10	5.60
	5.	G.W.	4.16	6.22	2.15	3.14	--	4.16	2.76
	6.	L.B.	11.63	4.81	2.72	3.42	--	2.08	5.46
	7.	R.D.	7.71	7.79	3.77	4.56	5.84	1.55	2.86
	8.	S.G.	1.85	2.40	3.82	--	--	1.96	5.26
Experimental Group Post-Training	1.	J.K.	10.23	6.00	--	--	--	10.25	8.48
	2.	D.C.	2.76	7.60	7.88	--	--	1.70	3.69
	3.	J.S.	4.40	2.07	5.40	4.38	--	1.34	8.80
	4.	D.I.	15.92	11.95	8.58	6.81	--	4.53	4.06
	5.	G.W.	3.35	6.00	2.84	3.28	--	7.53	6.91
	6.	L.B.	4.93	5.53	8.31	3.54	6.78	8.89	8.38
	7.	R.D.	6.88	10.93	8.35	--	--	6.42	13.90
	8.	S.G.	6.00	2.96	3.30	2.86	--	5.37	7.79

* measured as μmoles labelled glucose incorporated/min/mg tissue

TABLE 7

PHOSPHORYLASE b ACTIVITY* DURING SUBMAXIMAL EXERCISE

		Subject	Initial	20 min	40 min	60 min	80 min	Final	Recovery
Control Group	1.	B.H.	2.94	8.17	1.00	--	--	2.64	2.12
	2.	B.K.	1.48	1.85	--	--	--	2.78	1.88
	3.	G.R.	1.02	3.92	1.29	1.59	--	0.80	1.11
	4.	L.Do.	0.51	3.11	3.90	2.45	2.29	1.01	3.53
	5.	L.Du.	1.71	6.71	7.86	--	--	5.79	6.14
	6.	G.V.N.	1.60	1.91	3.26	2.78	0.89	6.87	1.35
	7.	L.W.	2.24	5.38	3.30	3.79	--	5.88	0.75
Experimental Group Pre-Training	1.	J.S.	4.50	2.87	2.78	4.19	4.50	2.73	2.83
	2.	D.I.	1.55	3.43	2.28	1.42	3.74	0.32	1.48
	3.	G.W.	2.01	0.67	3.20	1.23	--	0.96	2.11
	4.	R.D.	3.20	4.59	6.48	1.51	0.81	0.15	0.26
	5.	S.G.	0.42	0.22	0.41	--	--	0.37	0.55
Experimental Group Post-Training	1.	J.S.	3.63	1.90	7.19	4.71	--	6.26	1.98
	2.	D.I.	5.45	4.41	2.58	3.25	--	1.58	0.56
	3.	G.W.	0.45	0.46	2.89	--	--	2.91	2.48
	4.	R.D.	4.91	3.11	4.30	--	--	5.82	3.87
	5.	S.G.	1.64	3.92	2.57	0.07	--	1.86	0.44

* measured as μmoles labelled glucose incoporated/min/mg tissue

TABLE 8

TOTAL PHOSPHORYLASE ACTIVITY* DURING SUBMAXIMAL EXERCISE

	Subject	Initial	20 min	40 min	60 min	80 min	Final	Recovery
Control Group	1. B.H.	5.65	12.80	2.00	--	--	5.61	4.11
	2. B.K.	2.67	2.63	--	--	--	3.23	2.56
	3. G.R.	1.79	4.44	2.05	3.55	--	2.17	2.23
	4. L.Do.	4.16	7.15	6.61	4.30	2.64	5.17	7.54
	5. L.Du.	7.03	8.00	9.41	--	--	7.14	7.96
	6. G.V.N.	7.34	2.91	7.52	7.45	4.18	11.68	3.50
	7. L.W.	5.99	6.82	7.56	5.26	--	6.62	3.51
Experimental Group Pre-training	1. J.S.	10.92	4.32	6.05	6.45	6.44	6.25	6.62
	2. D.I.	11.48	7.92	6.96	4.30	11.36	5.42	7.08
	3. G.W.	6.20	6.89	5.35	4.37	--	5.12	4.87
	4. R.D.	10.91	12.38	10.25	6.07	6.65	1.70	3.12
	5. S.G.	2.27	2.62	4.23	--	--	2.33	5.81
Experimental Group Post-Training	1. J.S.	8.03	3.97	12.59	9.09	--	7.60	10.78
	2. D.I.	21.37	16.36	11.16	10.06	--	6.11	4.62
	3. G.W.	3.78	6.46	5.73	3.28	--	10.44	9.39
	4. R.D.	11.79	14.04	12.65	--	--	12.24	17.77
	5. S.G.	7.64	6.88	5.87	2.93	--	7.23	8.23

* measured as µmoles labelled glucose incorporated/min/mg tissue

TABLE 9

PHOSPHORYLASE *a* ACTIVITY* DURING MAXIMAL EXERCISE

	Subject	Initial	Final	Recovery
Control Group	1. B.H.	7.93	7.20	7.17
	2. B.K.	2.93	4.23	3.54
	3. G.R.	0.64	0.94	0.30
	4. L.Do.	10.67	9.67	12.81
	5. L.Du.	4.27	2.93	4.69
	6. G.V.N.	5.65	2.93	5.27
	7. L.W.	8.72	4.14	6.01
Experimental Group Pre-Training	1. J.K.	7.41	2.14	5.70
	2. D.C.	2.50	2.09	1.84
	3. J.S.	1.97	0.95	1.62
	4. L.B.	2.28	2.96	1.02
	5. R.D.	3.04	3.63	2.78
	6. S.G.	2.41	2.49	2.43
Experimental Group Post-Training	1. J.K.	16.11	8.32	8.47
	2. D.C.	4.56	2.45	3.33
	3. J.S.	9.23	5.88	6.06
	4. L.B.	4.82	5.56	3.43
	5. R.D.	7.05	6.49	11.12
	6. S.G.	4.42	5.35	7.41

* measured as μmoles labelled glucose incorporated/min/mg tissue

TABLE 10

PHOSPHORYLASE b ACTIVITY* DURING MAXIMAL EXERCISE

	Subject	Initial	Final	Recovery
Control Group	1. B.H.	0.30	6.90	5.78
	2. B.K.	0.86	0.13	1.32
	3. G.R.	1.61	1.15	2.61
	4. L.Do.	2.60	1.65	9.78
	5. L.Du.	1.66	6.16	2.61
	6. G.V.N.	12.04	1.63	6.35
	7. L.W.	5.13	4.31	6.40
Experimental Group Pre-Training	1. D.C.	1.19	1.15	2.36
	2. J.S.	6.05	1.91	5.91
	3. D.I.	6.71	5.53	3.54
	4. L.B.	9.11	6.73	7.75
	5. R.D.	1.93	1.20	0.83
	6. S.G.	2.83	1.19	0.21
Experimental Group Post-Training	1. D.C.	9.56	8.81	6.19
	2. J.S.	2.31	7.71	7.52
	3. D.I.	0.43	2.09	0.87
	4. L.B.	8.64	8.54	8.10
	5. R.D.	4.09	3.50	3.62
	6. S.G.	3.27	2.09	0.91

* measured as µmoles labelled glucose incorporated/min/mg tissue

TABLE 11

TOTAL PHOSPHORYLASE ACTIVITY* DURING MAXIMAL EXERCISE

	Subject	Initial	Final	Recovery
Control Group	1. B.H.	8.23	14.10	12.95
	2. B.K.	3.79	4.36	4.86
	3. G.R.	2.25	2.09	2.91
	4. L.Do.	13.27	11.32	22.59
	5. L.Du.	5.93	9.09	7.30
	6. G.V.N.	17.69	4.56	11.62
	7. L.W.	13.85	8.45	12.41
Experimental Group Pre-Training	1. D.C.	3.69	3.24	4.20
	2. J.S.	8.02	2.86	7.53
	3. L.B.	11.39	9.69	8.77
	4. R.D.	4.97	4.83	3.61
	5. S.G.	5.24	3.68	2.64
Experimental Group Post-Training	1. D.C.	14.12	11.26	9.52
	2. J.S.	11.54	13.59	13.58
	3. L.B.	13.46	14.10	11.53
	4. R.D.	11.14	9.99	14.74
	5. S.G.	7.69	7.44	8.32

* measured as μmoles labelled glucose incorporated/min/mg tissue

APPENDIX C

STATISTICAL PROCEDURES

Estimation of Missing Values

One requirement of repeated measures designs is that observations be available for each subject on each repeated measure. All the missing values in the present study were estimated by the iterative procedure proposed by Yates (84) and recommended by Federer (23: 126). For a missing value x_{ij} in row i and column j of a matrix:

$$x_{ij} = \frac{cx_{.j} + rx_{i.} - x_{..}}{(r-1)(c-1)}$$

where c = number of columns

r = number of rows

$x_{.j}$ = the sum of the (r-1) values in column j

$x_{i.}$ = the sum of the (c-1) values in row i

$x_{..}$ = the sum of the rc-1 observations in the matrix.

If more than one value was missing, guessed estimates were obtained for each. Each value, in turn, was then estimated by means of the formula. The cycle was then repeated for all missing values until the computed values became stabilized. Three cycles were usually sufficient, depending upon the closeness of the original guessed estimates.

This formula is such that each substituted figure is its own expected value, and so the error sum of squares is minimized (23).

Basic Design I: Repeated Measures on One Factor (81: 303-319)
Control vs. Pre-Training

Group	Subject	Initial	Final	Recovery
Control	1			
	2			
	.			
	.			
	n_1			
Experimental Pre-Training	1			
	2			
	.			
	.			
	n_2			

In this design, n_1 was not equal to n_2 in all cases, so an unweighted-means analysis was used (81: 375-378). Error variances were calculated using the data from all subjects, but the mean squares for groups, treatments and group x treatment interaction were derived by using only the cell means.

Basic Design II: Repeated Measures on Both Factors (81: 319-337). Pre. vs. Post-Training

	Pre-Training			Post-Training		
Subject	Initial	Final	Recovery	Initial	Final	Recovery
1						
2						
.						
.						
n						

Analysis of Variance Summary Table for Design I: Control vs. Pre-Training

Source of Variation	SS	df*	MS	F
Between Subjects				
Groups		$(g-1)$		
Subjects (Groups)		(n_1+n_2-2)	error(a)	
Within Subjects				
Treatments		$(t-1)$		
Groups x Treatments		$(g-1)(t-1)$		
Subjects (Groups) x Treatments		$(t-1)(n_1+n_2-2)$	error(b)	

For Groups main effect, $F = \frac{\text{MS for Groups}}{\text{error(a)}}$

For Treatments main effect, $F = \frac{\text{MS for Treatments}}{\text{error(b)}}$

For Groups x Treatments interaction, $F = \frac{\text{MS for Groups x Treatment}}{\text{error(b)}}$

Error (a) and error (b) are pooled error terms. No tests were made for homogeneity within these error terms.

* g = number of groups = 2

t = number of treatments = 3

n_1 = number of subjects in control group

n_2 = number of subjects in experimental group

Analysis of Variance Summary Table for Design II: Pre vs. Post-Training

Source of Variation	SS	df	MS	F
Between Subjects		n-1		
Within Subjects				
Groups		g-1		
Groups x Subjects	SS(a)	(g-1)(n-1)	error(a)	
Treatments		t-1		
Treatments x Subjects	SS(b)	(t-1)(n-1)	error(b)	
Groups x Treatments		(g-1)(t-1)		
Groups x Treatments x Subjects	SS(ab)	(g-1)(t-1)(n-1)	error(b)	

For Groups main effect,

$$F = \frac{\text{MS for Groups}}{\text{error(a)}}$$

For Treatments main effect,

$$F = \frac{\text{MS for Treatments}}{\text{error(b)}}$$

For Groups x Treatments interaction,

$$F = \frac{\text{MS for Groups x Treatments}}{\text{error(ab)}}$$

Conservative Degrees of Freedom for Critical F

In both designs and in all multiple comparisons, a conservative test proposed by Greenhouse and Geisser (30) was used to obtain the degrees of freedom for the critical F ratio. This conservative test makes maximum allowance for the danger, inherent in all repeated measures designs, of inequality and heterogeneity of variance-covariance matrices in the population. Between subjects effects are not susceptible to this source of error and so the degrees of freedom for these effects are not changed.

The degrees of freedom for all sources of variation within subjects must be multiplied by a factor,

$$\varepsilon = \frac{1}{(k-1)}$$

In this way the degrees of freedom for the critical F are reduced and fewer significant results are obtained.

	Effect	Normal df for Critical F	Conservative df for Critical F
Design I	Groups	$(g-1), (n_1+n_2-2)$	$(g-1), (n_1+n_2-2)$
	Treatments	$(t-1), (t-1)(n_1+n_2-2)$	$1, (n_1+n_2-2)$
	Groups x Treatments	$(g-1)(t-1), (t-1)(n_1+n_2-2)$	$(g-1), (n_1+n_2-2)$
Design II	Groups	$(g-1), (g-1)(n-1)$	$1, (n-1)$
	Treatments	$(t-1), (t-1)(n-1)$	$1, (n-1)$
	Groups x Treatment	$(g-1)(t-1), (t-1)(n-1)$	$1, (n-1)$
	Pooled Error		$1, 3(n-1)$

Multiple Comparison Procedures

Multiple comparisons performed on the results were non-orthogonal and determined a priori. Tukey's w procedure* (77), a fixed interval range test, was used to test the significance of all comparisons. This is a fixed interval range test in the sense that the maximum range (k=6 cell means) is used in determining the critical value of the studentized range statistic (q_r) for each comparison. No size-ordering of the means is necessary. Since the maximum range is used each time, Tukey's test is quite stringent and fewer significant results are to be expected (81: 88).

In the Tukey's w procedure:

- standard error of a single mean = $\sqrt{\frac{\text{error MS}}{N}}$, where N is the number of observations from which each mean is derived,

- $q = \frac{\text{difference between means}}{\text{standard error}}$

The appropriate error mean square for each comparison is listed in the subsequent pages of this appendix. The critical q_r used to assess significance has k equal to six in each case and conservative degrees of freedom associated with the appropriate error term.

---

* Also called Tukey's HSD or Honestly Significant Difference Test

Error Terms for Multiple Comparisons under Design II:

Pre vs. Post-Training

Comparison*	Error MS	N	Conservative df for q_r
I_1-F_1	Pooled errors (b) + (ab)	n	2(n-1)
I_1-R_1	$= \frac{SS(b) + SS(ab)}{4(n-1)}$		
F_1-R_1			
I_2-F_2			
I_2-R_2			
F_2-R_2			
I_1-I_2	Pooled errors (a) + (ab)	n	2(n-1)
F_1-F_2	$= \frac{SS(a) + SS(ab)}{2(n-1)}$		
R_1-R_2			
$\bar{I}$ - $\bar{F}$	Pooled errors (b) + (ab)	n	2(n-1)
$\bar{I}$ - $\bar{R}$	$= \frac{SS(b) + SS(ab)}{4(n-1)}$		
$\bar{F}$ - $\bar{R}$			

* I = initial; F = final; R = recovery

Subscript 1 refers to pre-training group
2 refers to post-training group

APPENDIX D

RESULTS: TABLES OF MEANS

TABLE 12

MEANS FOR PHOSPHORYLASE a ACTIVITY DURING SUBMAXIMAL EXERCISE

		Percent of Exercise Time					10 minute
Group	n	Initial	25%	50%	75%	Final	Recovery
Sedentary	7	3.30*	2.13	2.40	1.63	2.26	2.08
		±0.72	±0.51	±0.58	±0.38	±0.65	±0.41
Pre-Training	8	6.26	4.04	2.97	3.84	3.30	4.63
		±1.19	±0.65	±0.26	±0.51	±0.46	±0.45
Post-Training	8	6.81	6.71	6.18	5.71	5.75	7.75
		±1.54	±1.17	±0.85	±0.71	±1.13	±1.12

* means ± standard error of the mean

TABLE 13

MEANS FOR PHOSPHORYLASE b ACTIVITY DURING SUBMAXIMAL EXERCISE

		Percent of Exercise Time					10 minute
Group	n	Initial	25%	50%	75%	Final	Recovery
Sedentary	7	1.64*	4.13	3.50	2.87	3.68	2.41
		±0.30	±0.72	±0.75	±0.81	±0.92	±2.71
Pre-Training	5	2.34	2.40	2.57	2.04	0.91	1.45
		±0.70	±0.88	±0.67	±0.75	±0.48	±0.48
Post-Training	5	3.21	2.84	3.54	3.12	3.67	1.87
		±0.96	±0.73	±0.97	±0.84	±0.99	±0.64

* means ± standard error of the mean

TABLE 14

MEANS FOR TOTAL PHOSPHORYLASE ACTIVITY DURING SUBMAXIMAL EXERCISE

		Percent of Exercise Time					10 minute
Group	n	Initial	25%	50%	75%	Final	Recovery
Sedentary	7	4.95*	6.26	5.90	4.50	5.95	4.49
		±0.81	±1.06	±0.99	±0.81	±1.17	±0.87
Pre-Training	5	8.36	6.74	5.76	6.14	4.16	5.50
		±1.80	±1.56	±0.76	±1.02	±0.90	±0.70
Post-Training	5	10.52	9.43	9.74	7.58	8.72	10.16
		±2.99	±2.34	±1.65	±1.91	±1.13	±2.16

* means ± standard error of the mean

TABLE 15

MEANS FOR PERCENT PHOSPHORYLASE a ACTIVITY DURING SUBMAXIMAL EXERCISE

		Percent of Exercise Time					10 minute
Group	n	Initial	25%	50%	75%	Final	Recovery
Control	7	62.82	34.80	60.46	80.88	40.25	48.80
		±6.85	±5.28	±7.96	±10.71	±10.11	±7.33
Pre-Training	5	73.01	67.25	58.65	68.26	81.39	75.04
		±4.95	±9.79	±9.23	±10.28	±6.68	±7.70
Post-Training	5	70.96	66.97	59.55	75.26	58.12	83.19
		±6.33	±8.80	±6.52	±10.14	±10.92	±3.69

TABLE 16

MEANS FOR PHOSPHORYLASE a ACTIVITY

DURING MAXIMAL EXERCISE

Group	n	Initial	Final	10 minute Recovery
Sedentary	7	5.83*	4.58	5.68
		±1.33	±1.11	±1.45
Pre-Training	6	3.27	2.38	2.57
		±0.84	±0.37	±0.68
Post-Training	6	7.70	5.68	6.64
		±1.85	±0.78	±1.23

* mean ± standard error of the mean

TABLE 17

MEANS FOR PHOSPHORYLASE b ACTIVITY

DURING MAXIMAL EXERCISE

Group	n	Initial	Final	10 minute Recovery
Sedentary	7	3.46*	3.13	4.98
		±1.55	±1.00	±1.12
Pre-Training	6	4.64	2.95	3.43
		±1.27	±1.02	±1.20
Post-Training	6	4.72	5.46	4.53
		±1.48	±1.32	±1.31

* mean ± standard error of the mean

TABLE 18

MEANS FOR TOTAL PHOSPHORYLASE ACTIVITY

DURING MAXIMAL EXERCISE

Group	n	Initial	Final	10 minute Recovery
Sedentary	7	9.29*	7.71	10.66
		±2.18	±1.61	±2.47
Pre-Training	5	6.66	4.86	5.35
		±1.38	±1.25	±1.19
Post-Training	5	11.59	11.28	11.54
		±1.13	±1.22	±1.20

* mean ± standard error of the mean

TABLE 19

MEANS FOR PERCENT PHOSPHORYLASE a ACTIVITY

DURING MAXIMAL EXERCISE

Group	n	Initial	Final	10 minute Recovery
Control	7	64.20	60.56	50.46
		±9.57	±8.77	±7.55
Pre-Training	5	43.90	54.22	49.20
		±9.53	±9.30	±15.52
Post-Training	5	53.77	48.27	54.77
		±8.87	±9.07	±11.67

TABLE 20

MEAN GLYCOGEN LEVELS DURING MAXIMAL EXERCISE

Group	n	Initial	Final	Recovery
Control	7	0.76	0.69	0.71
		±0.05	±0.05	±0.09
Pre-Training	6	1.03	0.71	0.92
		±0.17	±0.15	±0.24
Post-Training	6	1.36	0.95	1.14
		±0.21	±0.14	±0.26